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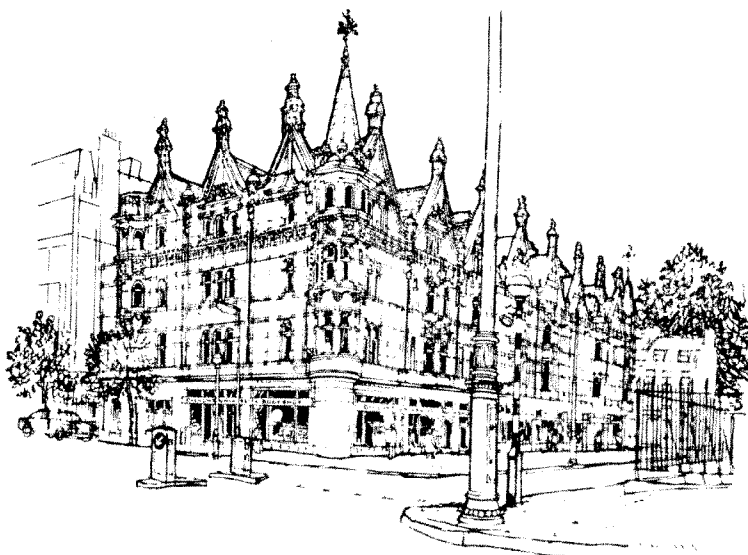
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### Cover picture

The discovery of an ear ossicle of *Australopithecus robustus* is reported on page 62. The dissimilarity between this bone and that of *Homo sapiens* exceeds that observed between *Homo sapiens* and the African apes.

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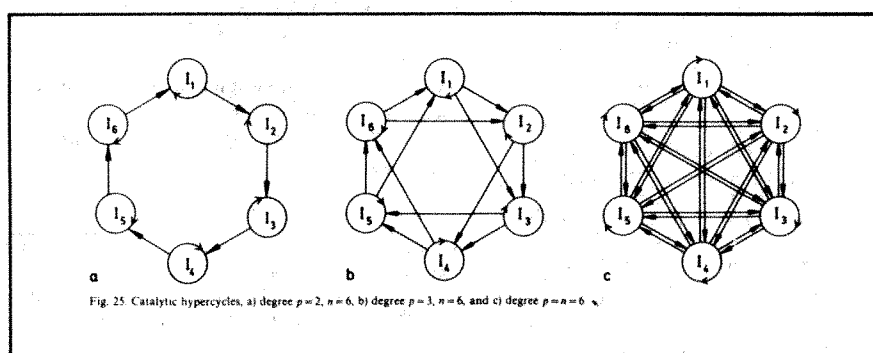
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# The Hypercycle

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Written with chemists, biochemists, biologists and physicists in mind, this book provides a deeper understanding of the origin and evolution of life, and will furnish the basis for many new experiments.



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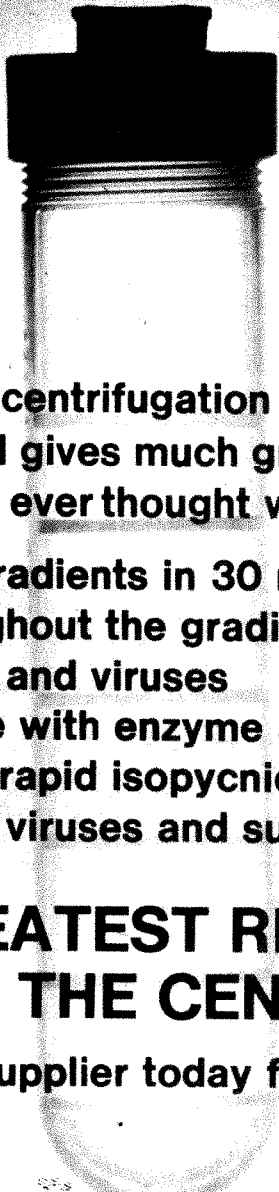
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## Costs and benefits of carbon dioxide

THE release of carbon dioxide to the atmosphere by the burning of fossil fuels is, conceivably, the most important environmental issue in the world today. Whatever direction global energy policies take in the future, it is indisputable that carbon dioxide concentrations in the atmosphere will continue to rise. There is still uncertainty about the ultimate destination of carbon dioxide. It seems that roughly half the fossil fuel output has remained in the atmosphere, and early workers supposed that the remainder was consumed by the oceans and the biosphere. But the role of the biosphere is now a matter of hot debate. Some research has suggested that far from being a sink for carbon dioxide, the biosphere (through deforestation and changing land use) could actually be a source. Other work suggests the contrary, or that the role of the biosphere has actually changed with time. But it is inescapable that atmospheric concentrations have already climbed by 15% as a result of man's activities during this century and there seems little doubt that concentrations would be double present values around the middle of the next century if current growth rates for the use of fossil fuels (over 4% per annum) were to persist. This is unlikely, of course, given the depletion of energy resources, but at least the figure gives some sort of guide for realistic modelling.

Whatever the uncertainties about future emissions and the biosphere, there is no disagreement amongst researchers on the qualitative impact that an increase in carbon dioxide will have on climate: mean annual surface temperature will rise, and the rises will be greater at high latitudes. There is also consensus that the hydrological cycle would become more active—with precipitation and evaporation levels both rising. Beyond this there is still scope for quantitative disagreement, but a commonly quoted figure is that a doubling of atmospheric carbon dioxide would result in a world global annual mean surface temperature rise of 2 to 3 °C, with marked latitudinal asymmetry. As yet, however, no model adequately accounts for changes in the ice-covered regions of the world or in the hydrosphere (particularly ocean currents), and there is considerable room for disagreement regarding the importance of feedback effects arising from changes in cloud cover.

With so much uncertainty around, is it irresponsible

and premature to widen the debate at this stage from meteorologists and climatologists to those with interests in the consequences of climatic change—agriculturalists, glaciologists, oceanographers, economists, sociologists, political scientists and so on? Surely not, provided that sensible perspectives are maintained. A recent workshop jointly sponsored by the American Association for the Advancement of Science and the US Department of Energy has been attempting to lay transdisciplinary foundations for a federally supported research programme on the impact of increasing atmospheric carbon dioxide content and it is not too early for other nations (or more reasonably groups of nations, such as the European Economic Community) to take similar initiatives. Even if large amounts of money were not immediately forthcoming, there are still some links across the specialist boundaries which ought to be made now.

In the long run the United Nations presumably has to get in on the act, and the United Nations Environmental Programme will shortly be setting up a carbon dioxide committee. At first sight scientists might despair at the thought of yet another area in which there will be politicised conflict between industrialised nations, large-scale releasers of carbon dioxide, and the developing world, involuntary recipients of the consequences. But careful reading of what climatologists and meteorologists have to say by way of prediction makes it clear that there could be as many benefits as losses as a result of temperature and rainfall changes—and that some parts of the world may even become cooler.

There is no clear indication that the animal and plant kingdoms will as a whole prosper more or less in a changed climate. And there may be direct carbon dioxide effects, such as changes in the rates of photosynthesis and respiration, increases in the efficiency of plant water use and changes in nitrogen fixation rates. To be sure, the most widely publicised effect of a substantial global warming is the danger of the West Antarctic Ice Sheet breaking loose and melting, with highly predictable effects on sea level. But for the rest the picture is complex and by no means universally gloomy. The sooner some of the complexities are unravelled, the sooner the carbon dioxide problem can be intelligently injected into discussions of world energy strategies. □



# President Carter aims to spend 'windfall' oil profits on energy research

PRESIDENT Carter last week asked members of the National Academy of Sciences to support his proposal that Congress create an Energy Security Fund, financed by a tax on the 'windfall' profits which the oil companies are expected to make following the de-control of oil prices. Such a fund could partly be used to double US investment in energy technology over the next few years.

The President's remarks were made during the course of an address to the annual meeting of the academy, the first to have been delivered to the assembled scientists since President John Kennedy spoke at a NAS meeting one month before his assassination. The Energy Security Fund, he said, which Administration officials estimate could involve collection from the oil companies of up to \$7 billion a year, would be used not only to provide relief to those least able to pay for more costly energy, but also to finance projects, including both basic and applied research, important to the country's energy future.

"By the second quarter of the 21st century, we will have learned to rely on cleaner, essentially inexhaustible sources of energy. The principal candidates include, of course, fusion and solar technologies as photovoltaics," President Carter said.

"We are preparing right now for these stages of our energy future. Our energy research and development is already larger in its programme size than those of all our allies combined. But we must do more."

The President had particularly harsh words for the oil industry, which is suggesting that the extra money it receives from increased oil prices should be used to finance its own energy development programmes, covering both exploration and research. He accused the companies of trying to 'hoodwink' the American people by passing a windfall profits tax that was in fact designed to provide the companies with loopholes through which they could increase their general revenues.

"They will try to pass this charade off on the American people as a so-called 'plough-back' provision. But it is not a 'plough back'—it is a 'plough under' and 'kick back'. And what is going to be ploughed under is the Energy Security Fund with its aid to research and to the poor," the President said.

"I ask for your support in the battle to pass an honest windfall profits tax . . . and I also call on all of you

in the scientific and engineering community to fulfil the trust of the American people by creating the new energy technologies that are so vital to the future of our country."

Although many observers in Washington feel that the President's idea of a windfall profits tax has a greater chance of succeeding in Congress than when it was first suggested last month, some have expressed doubts whether the amount of money that the Federal Government could collect in this way would in fact be as high as the President has suggested.

A report by Ralph Nader's Tax Reform Research Group has said that the proposed tax would pick up "only \$3.4 billion of the new revenues, ie, 20% rather than 50% of the increased revenues that the oil companies are expected to receive.

However the chances of Congress accepting the idea have been increased by the large profit increases which the major oil companies have been reporting for the first quarter of 1979. Texaco Inc., for example, reported an 81% increase in profits over the same period last year, and Gulf Oil Corporation a 61% increase. A spokesman for the President said that these profit increases greatly strengthened the arguments for a windfall profits tax to be used for research and other purposes.

In his speech to the academy, President Carter also asked the scientists to support his attempts to have the new Strategic Arms Limitation Treaty (SALT II), currently in the closing stages of negotiation with the Soviet Union, ratified by the US Senate.

Of all the fruits of science none is more bitter than nuclear weapons. And of all the responsibilities of nations, none is more urgent than the control of this most terrible menace to our



lives and to our civilisation," he said.

Many of the issues involved in assessing the treaty were very complex technically, and the American people would look to the scientific community to help shape an educated public debate. "Many of you devoted much effort to the debate over SALT I, and you played a major role in forming the consensus that developed to support that treaty. Today I ask for a renewal of that commitment."

The President also referred to the increasing costs of major scientific experiments—in contrast to the situation facing scientists such as Albert Einstein who required "little more than a few sharpened pencils and a quiet place to think"—and the choice that this posed between doing experiments on one's own, or in co-operation with other countries.

"We must continue to choose cooperation—for reasons that go beyond the considerable benefits of sharing costs and sharing ideas," the President said. "With our traditional friends, scientific and technological cooperation can strengthen existing bonds. With others, who may not be so friendly, it can help to bridge political, ideological and cultural division."

David Dickson

## More US accidents in transporting chemicals

THERE has been a sharp rise in the numbers of accidents and deaths involving the transportation of hazardous materials such as liquefied natural gas, sodium sulphhydrate, anhydrous ammonia and chlorine, according to a report published last week in Washington by the research service of the Library of Congress. According to the report, there were 45 deaths and 1,411 injuries involving the transport of hazardous materials in 1978, compared to 32 deaths and 749 injuries in 1977 and an average of 21 deaths and 592 deaths over the previous

period. The high accident rates in 1978 had partly reflected two major incidents, the explosion of a tank-car in Waverly, Tennessee, which killed 15 persons and injured 48 others, and the derailment of a goods wagon in Youngstown, Florida, which resulted in eight deaths and injured 158. The report says that present federal inspections may not be adequate, citing the fact that the Department of Transportation's Material Transportation Bureau has only six full-time inspectors to cover 20,000 container supply firms and 100,000 shippers. □

## Critics challenge data that led to 2,4,5-T ban

THE recent decision by the US Environmental Protection Agency to issue an emergency order banning the use of the herbicide 2,4,5-T has attracted strong criticism from its manufacturers. They are contesting the EPA's decision in a Michigan district court, claiming that it is based on a "seriously flawed study" and a misreading of data by the EPA to draw conclusions to support the ban.

The ban on 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) was made when a study in the Alsea region of Oregon found a strong correlation between the use of the herbicide and a tripling in the rate of spontaneous abortions among local women. The study was conducted by Dr Eldon Savage of the Epidemiological Studies Programme of Colorado State University and commissioned by the EPA. It was the second investigation into the health effects of 2,4,5-T on residents.

The first study—"Alsea I"—was inconclusive, according to the EPA, and led to the conclusion that the claims of Alsea women that their miscarriages were due to 2,4,5-T "had not been demonstrated from the data presented." However, the spontaneous abortions did appear to follow a seasonal pattern and 10 out of the 13 cases under investigation occurred between April and September; whereas hospital records indicated that increases in spontaneous abortions normally occurred in January-March and October-December.

Alsea II was designed to eliminate any element of bias which may have occurred in the first study, and covered a much larger sample. A 1,600 square mile rural area where 2,4,5-T spraying had taken place was chosen, with another 1,000 mile rural area and an urban area as controls. Data on spontaneous abortions in the first 20 weeks of pregnancy was taken from the records of hospitals in these areas.

The study revealed that the spontaneous abortion index (ratio of abortions/live births) was higher in the study area than in the other rural area. For the months of May, June, July and August, the index for the study areas was 89.9, 130.4, 105.4 and 88.1 compared with 63.2, 46.0, 55.3 and 79.8 for the rural control. In the urban area ratios were even lower. The investigation also showed a significant correlation with the 2,4,5-T spray pattern with a lag period of two to three months. The residents, the study suggests, could have come into contact with the herbicide and its dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) contaminant in drinking water or through eating fish or other wildlife.

One critic of the Alsea II findings is Professor Nathan Mantel, a statistician at the biostatistics centre of George Washington University, Bethesda. He claims there are "fundamental statistical and logical flaws" in the report. Mantel was asked to review the report by the National Forest Products Association (2,4,5-T is in widespread use in forestry) and his comments have been forwarded to the EPA. He says the EPA were wrong to compare results for a rural study area and an urban control. His analysis revealed no statistically significant differences between the data taken from the study area and a third "control" area.

Referring to the Alsea findings of a correlation between spray times and the seasonal pattern of abortions, Mantel insists that the comparison was based on an improper statistical approach. He claimed this result was of little value anyway, since there was no statistically significant difference in the overall rates of abortion in the areas investigated—the EPA study reported little difference in the percentage of hospitalised spontaneous abortions for women aged 20-49 in the three areas.

Concern about the EPA's misreading of data centres on its use of material from Seveso in Italy. Residents of this town were exposed to dioxin in July 1976 following an accident in a reactor manufacturing trichloropheno. With dioxin known from animal studies to be teratogenic there was concern that the chemical would cause malformation to increase in the Seveso children.

The EPA says this is precisely what happened, and that there was sevenfold increase between 1976 and the first five months of 1977. While admitting that the data collected at Seveso was inadequate, and that there were "methodologic deficiencies" in the way it was analysed, the EPA nevertheless says that the evidence provides "suggestive indications of a possible teratogenic effect [by dioxin] in humans".

Many scientists insist that the EPA is wrong on this point. The EPA has been sent evidence to show that the increase in malformations at Seveso is due to better reportage of these cases and that the increase is more apparent than real. The Italian Parliamentary Commission report on Seveso reached similar conclusions and pointed out that, while many scientists believed that dioxin was potentially teratogenic in humans, the evidence to confirm this point has so far not been found. Furthermore, if dioxin had been responsible for an increase in malformations at Seveso, then there should be evidence of an increase in specific defects. However, the data does not show this pattern.

The Michigan court hearing on 2,4,5-T will have to consider these conflicting claims. A decision to uphold the EPA ban will not have serious economic repercussions on the manufacturers of 2,4,5-T through loss of sales; it will leave them open to compensation claims from women who have miscarried.

**Alastair Hay**

## Windscale leak: Benn calls for public inquiry into safety

Mr Tony Benn, UK Energy Secretary, has called for a full public inquiry by the next government into the Windscale leak which was found last month during borehole sampling near a temporary storage tank for highly active liquid waste, dissolved in nitric acid prior to concentration and final storage. At a local Labour Party meeting on Saturday, Benn expressed concern about the present leak as well as about "the continuing and unsolved leak of less active substances that has been going on for several years." Benn asked the Nuclear Installations

Inspectorate whether Windscale should be closed but was advised that this step was unnecessary.

Friends of the Earth has asked Peter Shore, UK Environment Secretary, to conduct an inquiry into other Windscale incidents which include possible leaks from the low level waste burial ground, the discovery of  $^{137}\text{Cs}$  and  $^{60}\text{Co}$  on surface water drains, the closure of building B204 because of radiation hazard and the discovery last year of plutonium on a half incinerated dismantled cooling tower.

The outstanding problem of the

present leak is to determine the probable rate of migration of activity from the region of the borehole. Material which has continuously leaked from the sump has progressed to a distance of 10 metres in a year. With the leak stopped, future migration depends critically on the detailed site geology. Preliminary BNFL measurements indicate that an average migration rate is about a metre a year, but according to Friends of the Earth there has never been a full geological site survey at Windscale.

**Joe Schwartz**



# DESY makes a bid for protons in Hamburg

DESY, the West German subnuclear physics laboratory, appears to be establishing a rapprochement with CERN, Europe's international centre for the subject. It could rest on DESY building a superconducting storage ring for protons of around 280 GeV, while CERN builds LEP, a device for colliding 80 or 90 GeV electrons with positrons.

DESY would collide the protons with the electrons of its latest electron storage ring, PETRA, thus providing itself with the world's most precise instrument for probing the structure of the proton. LEP would be the most advanced tool for creating new forms of matter, such as new quarks, the intermediate vector boson, and Higgs particles.

This plan, which has yet to be formally adopted by the European high energy physics community, emerged at last week's inauguration ceremony for PETRA, held at the DESY laboratory in Hamburg. Professor Schopper, the director of DESY, told *Nature* that towards the end of this year he would be making an application to the German government to build a proton storage ring within the PETRA tunnel. The cost would be "about 1½ PETRAs"—around 150 million DM.

Earlier, the West German minister for science, Dr Volker Hauff, had made an extremely positive speech on behalf of basic science, from which it seemed clear that he would treat such an application sympathetically. "Political freedoms are inconceivable without a free science", he said unequivocally. However funds were limited (whereas research was unlimited) and choices had to be made. "We've got to limit ourselves to what we can do particularly well, where we can teach others and create top performance . . . with PETRA we do have a chance of creating this performance".

"With pride we can look forward to the development of PETRA as a centre of international research", said the minister. "More than half the researchers are guests, contributing their intellect and funds. PETRA is fundamentally enriched in this way."

And on the relationship of DESY and CERN, he said: "Very soon we will begin a dialogue with scientists to see if Germany can contribute to LEP. To have two flourishing laboratories, like CERN and DESY, is commensurate with the size of this continent. We cannot discuss the future of the one laboratory without thinking of that of the other."

This mood was reflected by Professor Jean Teillac, the President of CERN

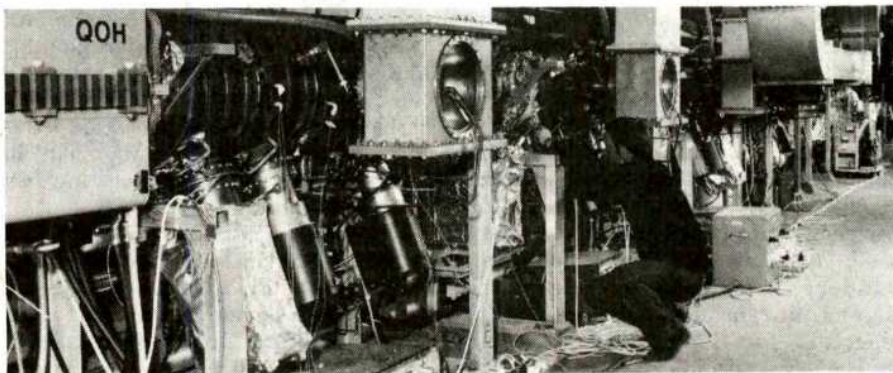
Council, the supreme body directing CERN, who said that "with CERN and PETRA Europe has a good, well-balanced programme . . . More even than in the past we must use central facilities like CERN and DESY".

Meanwhile, the new cooperative mood has yet to be translated into firm proposals by ECFA, the European Committee for Future Accelerators, which is currently deliberating on the

question of the precise energy for LEP—a delicate matter because costs increase rapidly with energy, particularly at CERN, where a big LEP would have to burrow under the Jura mountains.

"But we are not letting politics get in the way of our judgements", the chairman of ECFA, Professor Marcel Vivargent, said last week.

Robert Walgate



RF accelerator cavities at PETRA: resonances are causing beam losses

## PETRA in a race to highest energy

PETRA, the world's largest electron-positron colliding beam device, is still having trouble reaching design luminosity at its highest energy—and is beginning to fear the strength of the American competition, PEP, under construction at Stanford, California.

PETRA, the brightest jewel of West German sub-nuclear physics, is hoped to make two major discoveries: the mass of the sixth quark, "top" (or "truth"), and the first indication of the masses of the intermediate vector bosons (IVBs), the particles whose exchange is believed to be responsible for the weak nuclear force. But both experiments need the highest PETRA energies, and a reasonable data-taking rate.

Last week PETRA was brought up to 26 GeV, and 10 events—the results of collisions between 13 GeV electrons and 13 GeV positrons—recorded in two detectors. But the "luminosity" (effectively, the data rate) was a factor 70 below design, producing something of the order of one event per hour. At this rate the experiment to detect the IVBs—an ambitious construction by Nobel laureate Sam Ting—would take 50 years. "Top" might be found by chance, but really requires a systematic energy scan, which would also take a long time at current rates.

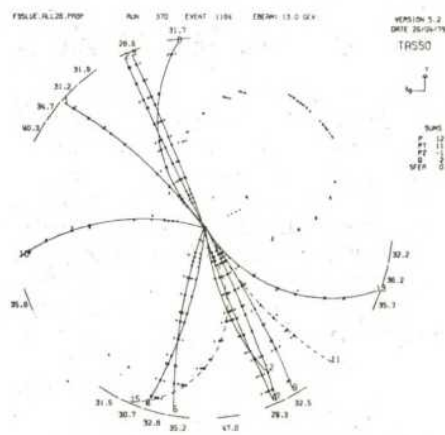
The problem appears to be due to the fact that PETRA must accelerate its own electron and positron bunches from 6.5 GeV, thus moving through a number of electromagnetic resonances in the 32 accelerating cavities. (Until now, at lower energies, PETRA

has used only four cavities.) The resonances make handling the beam a rather tricky procedure, and the beam is frequently lost.

PEP, on the other hand, which is due to come on line at the end of this year, takes injection from the Stanford linear accelerator at 17 GeV for positrons, and 24 GeV for electrons, thus scaling PETRA's "ramping" problems at one leap.

However a glass of water may be half full or half empty, and one's view of PETRA's difficulties depends on which side of the fence one sits. On the one hand the experimenters, such as Ting's group, appear to be pessimistic; but the accelerator physicists, whose job it is to produce the beam, hand-wave hopefully. Only time will tell who is right.

Robert Walgate



PETRA's first event at 26 GeV shows jet structure



# A Ukrainian line on science and education?

SCIENTIFIC research and education in the Ukrainian SSR is failing to meet the demands of the "scientific and technical revolution", according to Volodymyr V. Shcherbyts'kyy, first Secretary of the Central Committee of the Communist Party of Ukraine.

Addressing a meeting of university and college lecturers, Shcherbyts'kyy called for a "comprehensive attitude" to research, in terms which could be taken as a criticism of current Soviet policy.

Of recent years, policy has stressed the development of close contacts between academic institutions and local industry, with the scientists undertaking R & D on a contract basis as and when required. This, in Shcherbyts'kyy's opinion, is simply inadequate.

"Contracts between higher educational institutions and production are still essentially reduced to the conclusion of economic agreements with the neighbouring enterprises, and these agreements are frequently aimed at accomplishing particular, minor, tasks", he said. "A solution at the level of the entire branch practically never occurs".

What precisely Shcherbyts'kyy had in mind is not entirely clear. Presumably he would replace the present *ad hoc* trouble-shooting with a more structured programme of problem-orientated R & D, somewhat on the Polish model. Such a system, he im-

plied, would need to be organised on the regional level by the Ukrainian republic.

At present, he said, the Ukrainian Ministry of Education "has no joint plans with any [other] Ukrainian Ministry", although it is carrying out "several comprehensive plans" with the All-Union Ministry of the Chemical Industry. This lack of contact with production at the republic level, said Shcherbyts'kyy, is apparently why only about 20% of the inventions patented by scientists at higher educational institutions in Ukraine ever get applied in industrial production.

The relationship between the economies of the Union Republics and the central planning authorities is a complex one, and the relevant paragraphs of the new Soviet constitutions do little to clarify the matter.

Long ago in the 1950s Khrushchev tried to over-ride the republic boundaries with a system of arbitrary economic planning regions. Although this set-up has been abandoned, at least as far as education and academic life is concerned, there has been a constant trend towards centralisation in the last two decades.

Recently, scientific publication in languages other than Russian has virtually ceased, and several promising lines of research in individual republics disappeared into main-stream anonymity. The distinctive Ukrainian

school of cybernetics of the early 1960s was probably the most notable such loss. Young graduates on leaving university are drafted wherever the economy requires—generally to the new towns of Siberia.

To date Shcherbyts'kyy has shown no signs of Ukrainian national sentiment—indeed, he was appointed in 1972 to replace Shelest, who fell from power precisely for his overly-Ukrainian attitude.

So First Secretary Shcherbyts'kyy's advocacy of strengthening academic-production contacts at a republic level seems essentially a pragmatic response to a pressing economic need, not a criticism of Soviet "nationalities policy", which to date he has resolutely supported.

On educational policy, however, he does seem prepared to make a cautious criticism. After noting "frequent cases of formalism", "unproductive use of time" and "a peculiar percentage mainia" in assessing the knowledge of students", he went on to the "participation of pupils and students in construction detachments and in agricultural operations"—officially viewed as a major factor in communist education.

Although paying official tribute to the "importance" of such activities, he nevertheless found it "necessary to emphasise that studies must be the first objective to which the student must apply his efforts". **Vera Rich**

## Soviet soil erosion laboratory defends its economic record

Moscow University's laboratory of soil erosion and river bed processes is already saving the Soviet economy some 10 million roubles per annum. So said its director, Dr Roman S. Chalov, interviewed on Moscow radio last month on the tenth anniversary of the opening of the laboratory.

Appraisal of R & D in terms of pay-off and cost-effectiveness is a regular Soviet practice—although perhaps more common among policy-makers than scientists.

Dr Chalov's laboratory, however, is in a somewhat sensitive position. Last December, the Party Central Committee and the USSR Council of Ministers adopted a resolution "On additional measures to strengthen nature conservation and to improve the use of natural resource, and among the sectors singled out for special criticism, the resolution mentions soil erosion, which the planners note "is doing considerable harm to the national economy".

Dr Chalov's laboratory, being the only one in the Soviet Union specifically devoted to erosion problems, can hardly have taken this observation lightly. Chalov's anniversary interview accordingly became an apologia for the work of the laboratory. The 10 million per annum, he implied, was merely an earnest of what the laboratory hopes to do.

At present the team are largely concerned with feasibility studies. In the North Caucasus agricultural area, a study has been made of the economic efficiency of different anti-erosion measures, giving special attention to the "accelerated erosion" produced by agriculture. A further field study will be undertaken this year in the non-black soil zone of the Russian SFSR. Development of this region of relatively poor agricultural land has a special place in the current Five Year Plan. Here the team will study gully erosion—the destruction of the land by flash floods and torrents.

This extensive programme of anti-erosion work, actual and potential, has one rather surprising omission. It is entirely water-related, and has no concern with wind-erosion and desertification. This is the more surprising since Khrushchev's policy of horizon-to-horizon cultivation of the virgin lands has created a number of incipient dust-bowls.

Desert erosion, is the concern not of Moscow University, but of Leningrad. A major expedition from Leningrad University and the Main Geophysical Laboratory last summer made a detailed study of the formation and growth of dust-storms in Soviet Central Asia.

According to the teamleader, Dr Kiril Kondrat'ev, the main subject of study was the "anti-hothouse" effect, which occurs during dust-storms which extend to high altitudes, cooling the Earth's surface by reflecting solar radiation.

**Vera Rich**

# Third World to demand new fund for scientific development

THIRD World countries are making a strong bid to have the creation of a new fund for scientific and technological development occupy a prominent position on the agenda of the United Nations Conference on Science and Technology for Development (UNCSTD), which takes place in Vienna at the end of August.

The new fund is currently referred to as the International Science and Technology Development financing system. It is among the proposals submitted to the UNCSTD secretariat last week by the Group of 77—the organisation which represents over 100 developing countries—as a possible re-drafting of a proposed programme of action to be adopted by the conference.

At present there is still considerable disagreement among members of the Group of 77 over precisely what form the fund should take and how it should operate. However, some broad principles have been agreed, such as the fact that it should operate within the United Nations system, and that it should also provide relatively stable and continuous funding, to avoid problems for scientific programmes caused by intermittent or fluctuating support.

Other proposals that have been made are that the fund should be made up of contributions based on agreed percentage of the average deficit in the trade balance in manufactured goods between the developing and developed countries; that it be financed in part through a reduction in armaments expenditure by the developed countries; and also that further funding might come from a tax paid by developed countries to compensate for the brain-drain from developing countries.

The developed countries, however, have already made it known that they will be very reluctant in Vienna to accept any proposals which will require to provide substantial increases of funds. The issue is therefore likely to be one of the most hotly discussed during the conference.

The two other main issues likely to generate debate, it emerged from last week's meeting of the conference's preparatory committee in New York, are the development of appropriate institutional mechanisms—including the rearrangement of responsibilities for science and technology within the UN system—to support any policy recommendations made by the conference, and possibly some specific proposals for ways of stimulating collaborative research on topics of particular interest to developing nations.

The developing nations are more concerned at present about financial mechanisms and about institutional arrangements—the developed nations about specific research proposals. As things stand at present it seems unlikely that any major changes will emerge directly from the conference itself (restructuring of the UN system, for example, can only be done by the General Assembly). But there are still hopes that the conference will produce guidelines representing a consensus on how each of these three items should be approached.

The main development in the conference preparations since the last meeting of the preparatory committee in New York in February has been the efforts of the Group of 77 to develop a unified position which the developing nations can present at Vienna.

A working party of the Group of 77 met in New York for the first two weeks in April to prepare a set of amendments to the preliminary draft programme of action which has now been drawn up by the secretariat under three headings: strengthening the scientific and technological capacities of developing countries; restructuring the existing patterns of international scientific and technological relations; and strengthening the role of the United Nations in science and technology and the provision of increased financial resources.

In parallel to this effort the economic commission for Latin America organised a meeting of a group of experts in Lima, Peru, in March to discuss possible financing mechanisms to provide additional support for science and technology in developing countries,

making various proposals which, although not officially endorsed by ECLA, have been partially amalgamated into the Group of 77.

At the end of last week, the Group of 77 presented to the preparatory committee its suggestion on revising the first part of the draft programme of action, that which deals with internal arrangements within countries for developing science and technology.

Many of the suggestions such as the need to establish science and technology policies as part of general development policies, and to encourage cooperation between developing and developed countries, as well as between developing countries themselves, had already been made by the secretariat itself on the basis of the suggestions contained in the various national and regional papers which have been prepared and submitted as part of the planning process for this conference.

With regard to financial resources, the group of 77 says that present funding mechanisms for enhancing national science and technology systems "are highly unsatisfactory both from the qualitative and quantitative considerations" and adds that experience has shown that "there is need for a more effective financing system within the United Nations system for strengthening the science and technology capacities of developing countries".

In general the developing countries suggest that developed countries should devote on an annual basis 0.05% of their gross national product to the solution of scientific and technological problems of developing countries, and devote at least 10% of their R & D expenditure to programmes designed to solve problems of specific interests to developing countries.

More specifically they recommend the setting up of an International Science and Technology Development Financing System to provide financial resources to supplement national science and technological financing capabilities.

Precise details of how this system would work were still under discussion by the Group of 77 at the beginning of this week. In an earlier draft of possible recommendations prepared by the working party, however, it was suggested that any new system of financing be based on a number of features, including provision that money be made available on a predictable and continuous basis, and that it should be based on a linked to international economic parameters which reflect the present asymmetries between countries in technical capacity.

Appropriate provision should be made, the working party suggested, for the financing system to involve the use of regional funds, for which the ad-



"Every time they make a bomb, we make a killing!"

ministrative responsibility would be bestowed on the developing countries themselves, while overall responsibility would rest with the UN general assembly.

Detailed discussion on these various points are likely to occupy much of the time up to the Vienna conference. Meanwhile, in an attempt to make sure that something of a more immediately practical nature emerges from UNCSTD, various countries are sup-

porting proposals that specific efforts should be made to establish projects in areas requiring greater research. Belgium, for example, submitted a resolution to the preparatory committee last week suggesting five areas for international collaborative research: solar energy, rural development technologies, tropical diseases, methods of increasing food production, and an inventory of natural resources.

David Dickson

## Agricultural research centres transformed

THE dozen international agricultural research centres set up between 1959 and 1976 to conduct research into problems of tropical agriculture have had considerable influence on the national research systems of many developing countries as well as on the crops and methods used by farmers. The very changes the international centres have stimulated, however, have in turn resulted in changes being made to the mode of operation of the centres themselves so that they are no longer working to the rules laid down when the first, the International Rice Research Institute, was set up twenty years ago.

Speaking to a small gathering at the Science Policy Research Unit, University of Sussex last week, Dr Edward Clay of the university's School of African and Asian Studies said that the international centres had greatly influenced the way scientists in third world countries "perceive the research they're to carry out". In particular ever since IRRI and the International Center for the Improvement of Maize and Wheat (CIMMYT), the first two centres to be set up, achieved considerable success in breeding high-yielding dwarf varieties, most research on increasing yields for all sorts of crops in many countries has centred on finding dwarf varieties.

In India, for example, rice research has tended to focus on light-yielding semi-dwarfs for irrigated land. 67% of rice breeding is oriented towards irrigated rice, even though only 28% of the land used for rice cultivation is irrigated. And almost all the new varieties developed have been semi-dwarfs. Only 5% are suited for deep water although 40% of the land used for rice growing is under deep water.

According to Dr Clay, the international centres cannot be held entirely responsible for this mismatch of research to needs although it is highly likely that they have had some influence. In the case of rice research, for example, IRRI has mainly concentrated on irrigated rice—simply because of the local conditions around its home in the Philippines.

The centres' influence, however,

claims Dr Clay, extends beyond the individual scientist to the very agricultural policy of developing countries. "Experts were put into the international centres and were successful", he says "therefore the model of a centre was seen as a good recipe for success". More international centres were built and individual countries began to build their own single purpose centres. The Bangladesh government, for example, decided to create one specific rice research institute—"very large, costly and capital intensive with well-trained scientists". This was a new idea for an individual country—even countries with a highly organised research effort such as the US and Japan had not concentrated their research on just one topic into one place.

When IRRI and CIMMYT were first set up, they were intended to be solely research centres where Western scientists could apply certain techniques which had proved beneficial in agricultural research for temperate zone crops to tropical crops. As the influence of these first two centres spread, however, and individual developing countries began to take up their methods, they have played an increasing role in educating third world scientists and organising exchanges. With the creation of the Consultative Group for International Agricultural Research (CGIAR) "to raise funds and establish research policies" for the centres and the creation of more centres in different parts of the world, their operation has gradually become more bureaucratic.

"We now have well-defined bureaucratized institutions", according to Dr Clay. "This was not true of the original centres. In expanding and replicating the original model we've now got something quite different". There is now pressure for the centres to work much more with developing countries than was initially intended. And, according to Dr Clay, as they are essentially funded by industrialised countries this has put them into a complicated relationship between the aid-donors and their recipients.

Judy Redfearn

## Pre-UNCSTD colloquium planned

PREPARATIONS for the Colloquium on "Science, Technology, and Society" to be held by the UN Committee on the Application of Science and Technology to Development (ACAST) in Vienna next August immediately before UNCSTD, were the main item on ACAST's agenda when it met in Geneva recently. At the Colloquium 200 invited representatives of the world scientific and technological community will help formulate a document for discussion at UNCSTD.

During the first week of the ACAST meeting it became apparent that the Colloquium is seen only in terms of certain items of the agenda of UNCSTD. For whatever reasons, the members of ACAST seem to have forgotten their hitherto jealously guarded role as an independent advisory committee, and limited themselves mainly to points they had been asked to consider by UNCSTD Secretary-General, José da Costa.

However, da Costa himself possibly helped to harden ACAST's attitude. His personal intervention on a flying visit, during which he did not even stop to answer many of the questions he inspired, appeared to reflect considerable pessimism as to the outcome of his own Conference. Seeing less than a fifty-fifty chance of any concrete results, he feared it would be only a "conference verbale", within which ideas on, amongst other things, new forms of cooperation between North and South, as well as within the South might at best appear in the form of recommendations.

Da Costa's statement was also noteworthy for a sudden and highly emotional attack on the way in which his conference is being treated by the media, in particular by the "Lund Letter", published by an internationally-oriented group in Sweden, and which he singled out as "nordique et paranoïaque".

A second factor which further enlivened the ACAST proceedings was what looked remarkably like an attempt on the part of UNESCO, through their representatives at the meeting, to take over the whole Colloquium, albeit still disguised as an ACAST-sponsored meeting. Whether this was because of their frustration at the admittedly somewhat tardy and, to some observers, impractical way in which the Colloquium was being planned and organised, is not clear.

However, here the members of ACAST closed their ranks and refused to be stampeded by UNESCO's probably justified calls for urgency.

Peter Collins



## news in brief

**UN urged to take lead in studying effects of CO<sub>2</sub>:** The United Nations Environment Programme can and should take the lead in assessing the global impact of an increase in carbon dioxide on the environment and health and society generally, Mrs Barbara Blum, deputy administrator of the US Environmental Protection Agency, told UNEP's governing council in Nairobi last week. She also called for an international meeting of experts to accelerate and coordinate action to improve management of the world's forests. "Tropical forests are the world's richest generic reservoir, a potential source of useful plants and drugs, a modulator of climate, a shield against desertification and soil loss, and a renewable timber-bank", she said. "It is time to highlight this key problem for decision-makers at the highest level of government."

Recent experience in the US, which was now having to clean up the consequences of earlier indiscriminate dumping of toxic wastes, showed that the land was not an infinite sink or sponge which could be used as a dumping ground for residues of industrial activity.

**Russian invitation:** France has been asked to supply a cosmonaut for a space flight with a Soviet crew. This proposal was made by Mr Brezhnev to President Giscard d'Estaing, during the French leader's visit to Moscow last week. According to Tass, Giscard "responded positively".

**TUC gets in on microprocessors:** Two weeks after the UK Science Research Council jumped into the technology of microprocessors with proposals for a major research and training programme (19 April), the Trades Union Congress has urged full cooperation between government, management and the unions to avoid the loss of "hundreds of thousands of jobs". Mr Len Murray, TUC general secretary, notes in a report that "in the absence of intervention and planning, the benefits of microelectronics will certainly be unequally distributed". The TUC calls for a 35-hour week, a reduction of systematic overtime, longer holidays and sabbaticals and trade union education in microprocessor technology. But Professor Kristen Nygaard of the Institute of Informatics of the University of Oslo says that the key issue is one of workplace control. He cites the introduction of "data" shop stewards in Norway as a more appropriate demand for trade unions to make. The shop stewards serve on small shop floor committees of four to six persons and they are not permitted to become programmers. The stewards are also required to attend the annual data processing course given by the Norwegian TUC that has been held since 1971. *Employment and Technology. TUC, Great Russell St., London WC1. 40p.*

**Cornell ring starts storing electrons:** The first circulating beam of electrons has been stored at Cornell University's new Electron Storage Ring, a facility which, when fully operational later this year, will enable stored beams of electrons and positrons to collide at two points in their orbit. The storage ring is mounted in the same tunnel that houses the Cornell electron synchrotron, which will provide the high energy electrons and positrons for injection into the storage ring. Conversion of the synchrotron into a colliding beam facility has been under way since September 1977, and has been funded at an estimated cost of \$20.7 million by the National Science Foundation.

**Head of FDA resigns:** Dr Donald Kennedy has resigned as director of the US Food and Drug Administration, an agency of the Department of Health, Education and

Welfare, to return to Stanford University in California where he is to become provost and vice-president for academic affairs. Before joining the FDA in March 1977, Dr Kennedy was professor and head of the human biology programme at Stanford. For a time he was also presidential science adviser, acting as one of two senior consultants to the Office of Science and Technology Policy. Announcing his decision to return to academic life, Dr Kennedy said he had intended to stay at FDA through a full academic term, "but the position at Stanford will not wait". He leaves the FDA on June 30—one day before a tough new ethics law restricting the contacts that ex-government employees can have with their previous agencies comes into effect—and starts work at Stanford on August 1.

In his two years at the FDA, Dr Kennedy has been at the centre of a number of controversial issues. He has strongly supported attempts to ban saccharin as a potential carcinogen, and has strongly opposed moves to legalise the medical use of the claimed anti-cancer agent laetrile. Last year he was also behind unsuccessful attempts by the administration to overhaul FDA's drug-regulation system.

**New analysis traces consequences of world resource depletion:** Worldwatch Institute, a Washington-based non-profit research organisation, has released a study on the economic effects of population growth, which finds that significant per capita declines are occurring in four major resource areas: fisheries, forests, croplands and grasslands. Over-fishing has become the rule rather than the exception. In forestry, demand has exceeded the sustainable yield of the world's forests producing higher costs for lumber and a consequent shortage of housing. Grassland management is characterised by overgrazing on every continent and pressure on beef, milk, wool and leather production. Crops are also strained, and cereal production, which increased 30% from 1950 to 1971, has levelled off.

The study points out that when biology starts to fail, oil tends to be substituted, thus placing a drain on energy reserves. Synthetic fibres now account for a third of the market and synthetic rubber two-thirds. Cardboard and wood are replaced by plastics. In addition, agriculture depletion increases the demand for chemical fertilisers and mechanised agriculture to maintain production. Worldwatch says: "The oil safety valve is starting to stick." The study is critical of supply and demand solutions and sees a need for government intervention in population policy and resource management and increased research into the capacity of the world's biological systems.—*Resource Trends and Population Policy: A Time for Reassessment. Worldwatch Paper 29.*

**Sino-Bangla technical cooperation:** China and Bangladesh have signed an agreement for the mutual exchange of scientists and technicians to study agricultural production. China will organise study visits for Bangladesh experts in the freshwater fish farming, paddy rice culture, small irrigation projects and agricultural machinery manufacture. Also included will be the processing of green tea and Chinese medicinal herbs and visits to study the use of natural gas for producing chemical fertiliser. Bangladesh will offer study visits on jute manufacture, black tea growing and cultivation of mango saplings. About 50 Bangladeshis will visit China in the first group which will leave in the next two months. Bangladesh has been aligned towards Western technological development but the Chinese agreement may open up opportunities for the transfer of traditional technologies based on local resources.  
—from M. Kabir in Dacca.



# Multi-mirror telescope confounds the sceptics

**David Dickson** reports on a telescope with a radical new design which is to be officially inaugurated in New Mexico next week

FROM the main road it looks like a huge, gift-wrapped Christmas present, perched precariously on the summit of the 8,500-ft Mount Hopkins, 25 miles south of Tucson, Arizona. Only as you cover the last, steep stretch of the approach road and begin to glimpse the apparatus behind the rolled-back doors, does the building's function as an observatory become apparent—despite the absence of the conventional split dome.

Even then, the surprises are not over. Rather than the long, carefully balanced cylindrical telescope that one expects, there is a squat, almost cube-shaped construction with six identical mirrors hidden in a complex of steel girders, the whole apparatus supported by a massive steel yoke.

The Multi-Mirror Telescope, has been built jointly by the Smithsonian Institution and the University of Arizona, and is to be officially inaugurated next week. Attending the dedication ceremony may well be some of those who were initially sceptical about the feasibility of combining six separate reflecting mirrors in a single telescope, a step which the designers claim to be the first major departure from conventional optical telescope construction in more than a century.

"It is a radical design, and it met with an enormous amount of scepticism in the beginning. Even those involved in the project found they had to overcome some of their own biases and prejudices", says Professor William F. Hoffmann, of the University of Arizona's department of astronomy. "Many doubted whether it was practical to trade off the cost of a single, large primary mirror against the complexity of the control and alignment systems needed to operate a number of smaller mirrors simultaneously. And even if the trade-off proved to be worth it, there were doubts about whether the control system could be made to work adequately—and to run routinely."

Some of these reservations—and in particular the last one—have yet to be answered. But preliminary viewings have already been successful, even

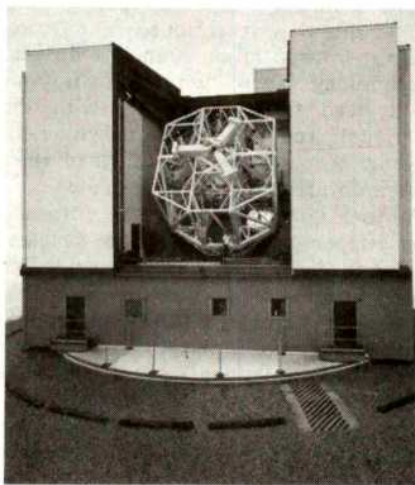
though the telescope has to be operated manually until the computerised control system is linked up. Professor Hoffmann says he does not foresee any major problems.

There are three radical innovations in the MMT design, each of which is being closely followed elsewhere as suggestion new directions in telescope construction. The MMT is really a combination of six parallel Cassagrain telescopes, each with its own 1.8-metre primary mirror and its own secondary mirror, with the separate images focused to a single point. This results in a light collecting capability equivalent to a 4.5-metre telescope—still behind the 5-metre telescope on Mount Palomar and the Russian 6-metre telescope, but its compact design means that, at \$7.5 million (in 1975 prices), the MMT cost less than a third of a conventional design.

Laser beams align the mirrors, using the MMT's guide-alignment telescope as a collimator for a laser-generated point source. The beam is split into six components, each used to keep a check on one of the mirrors and control its fine-adjustment mechanisms. "The problem is comparable to getting six headstrong ballerinas to dance as if they were one," says one of the project directors.

MMT may well serve as a prototype for a new generation of optical telescopes, avoiding the increasingly prohibitive costs of large primary mirrors. Already a multi-mirror design is being considered as one option for a 10-metre telescope being planned by the University of California. Scientists at the neighbouring Kitt Peak National Observatory are exploring a similar approach to a 25-metre instrument.

The second design innovation is the



Complex of steel girders hides the mirrors

mounting. Most conventional telescopes use an equatorial mount, meaning that movement about only one axis is required to compensate for the rotation of the Earth. The MMT, in contrast, uses an alt-azimuth mount, the horizontal axis being provided by a large steel yoke which itself turns on a vertical axis. Keeping track of an object during viewing means rotating the telescope about both axes simultaneously, a process which is controlled by a special mini-computer.

Both the compact design of the telescope, and the alt-azimuth mounting, led to the concept of a rectangular housing. "Any dome is, in its own geometry, azimuthal, but with an equatorially-mounted telescope you need the extra swing-space within the dome," says Professor Hoffmann. "With the alt-azimuth mount, motion is either up and down or circular, so saving space inside the dome is no longer necessary. A rectangular space then became much more convenient than a spherical one."

Professor Hoffmann says that although when first suggested the fork mounting and the rectangular building were considerable innovations, it turned out to be so practical that he thinks "people will soon find that the hemispherical dome and the equatorial mount are no longer attractive". Scientists from the Australian National University are already contemplating using this approach for a new telescope on Mount Stromlo, and others are expected to follow.

When it becomes fully operational, the MMT, which will be able to operate in both visible and infra-red parts of the spectrum, will have a number of uses. It will be able to carry out infra-red photometry that could help scientists identify materials on the surface of Pluto and the satellites of Uranus and Neptune, and also provide detailed measurements of highly red-shifted optical spectral lines from distant quasi-stellar objects.

Another major application will be in spectroscopy. By projecting the images from the six main mirrors along a line, rather than on to a single spot, the MMT will be able to compensate for the light lost when the single image from a conventional telescope is focused on a single slit.

With other ground-based facilities—particularly those at Kitt Peak—increasingly in demand, there will be no shortage of users for the MMT. "They are already beating a path to our door," says Professor Hoffmann. Initial users will be those from the Smithsonian and the University of Arizona. Subsequently, applications for experiments will be open to all comers. □



# 'North' meets 'South'—without the bickering



**Roger Revelle** reports on hopes for a new era of scientific and technical cooperation in the Third World

FOR a variety of reasons, including the probable makeup of the national delegations, the United Nations Conference on Science and Technology for Development (UNCSTD) to be held in Vienna in August, will be largely political and diplomatic.

So to provide a forum for scientists and engineers to express their ideas for UNCSTD, the International Council of Scientific Unions (ICSU) enlisted the cooperation of other international professional organisations (including those of the social scientists, humanists, physicians and engineers, and such quasi-scientific groups as the Club of Rome and Pugwash), in sponsoring an International Symposium on Science and Technology for Development, which was held in Singapore during the week of 22 January under the chairmanship of Thomas Malone, Treasurer of ICSU and Foreign Secretary of the National Academy of Sciences. Some 120 natural and social scientists and engineers from forty countries participated, besides observers from the United Nations and its specialised agencies, and from national technical assistance organisations. More than half the participants came from the developing countries of Africa, Asia, and Latin America, while the remainder represented both the capitalist and the 'centrally planned' industrialised countries. Notably absent were representatives of the People's Republic of China—which has had difficulties with ICSU over Taiwan—and the oil-rich Arab countries, though scientists from Egypt, Sudan and Morocco actively participated.



Thomas Malone

Some of the more important conclusions of the symposium were:

- The application of science and technology for development depends very largely on the existence of effective scientific and technical institutions within each developing country. Through these, each country can improve its indigenous technology,

make rational choices among foreign technologies, absorb chosen technologies, and create new ones appropriate for its special circumstances. These institutions will need to conduct some basic and applied research, as well as technological development, in order to retain a competent staff and to stay aware of scientific advances throughout the world that may eventually become useful to their country.

- To build up their scientific and technical capabilities and their industries, the developing countries require a greatly increased corps of qualified technicians and other skilled workers. A large number of technical schools and junior colleges should thus be established in the Third World, and should be open to both sexes to expand employment opportunities for women. Apprenticeship training for LDC technicians in the industries of the developed countries and in multinational corporations in their own countries should be strongly encouraged, while school curricula in science and technology need expansion and modernisation, with emphasis on experimental work and manipulation of equipment.

- The establishment of regional research and development institutions (analogous to the present international agricultural research centres) should be considered, covering a wide range of scientific and technical fields, including forestry, fisheries, management of land and water resources, construction, transportation, and industrial technology. These regional institutions will need to work closely with the national research and development agencies, to ensure the results of their research are put to practical use.

- More donor agencies concentrating on support of research and development in and for the Third World, like the International Development and Research Centre (IDRC) in Canada, the Netherlands University Foundation for International Cooperation (NUFIC), the Swedish Agency for Research Cooperation with Developing Countries (SAREC), and the Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) should be established

in the industrialised countries. Existing agencies should be encouraged to co-operate more closely with each other in exchanging information and ideas and in creating interacting programmes. The outstanding work of the International Foundation for Science and the United Nations University, in supporting applied research by LDC scientists within their own countries, deserves greater financial backing by the developed countries.

- Physical and biological scientists and engineers in the developed countries need to be more aware of the developing countries' underlying problems, whose solution demands both basic and applied research; they should be encouraged to undertake such research in cooperation with scientists from the Third World. For this purpose, the international scientific organisations need increased participation by scientists of the developing world, and improvements in their analysis and communication in areas related to developing country problems. UNESCO and other specialised UN agencies could help the non-governmental organisations play a more effective role. International 'workshops' focused on specific problems, in which scientists from developed and developing countries jointly formulate research and development programmes, can serve as a catalyst for cooperative efforts.

One distinguishing aspect of the symposium was the range of talent, experience and knowledge among the participants; another was the absence of the harsh confrontations between the "North" and the "South" that have frustrated useful discussion in many recent UN-sponsored conferences. Instead, it seemed clear at Singapore that a new international scientific and technical order is an essential prerequisite for the new international economic order so earnestly desired by the countries of the Third World.

The symposium considered ways in which science and technology could be used to alleviate some of the most critical developing country problems. The real incomes of the poorest classes need to be increased, by reducing unemployment and increasing productivity by means of appropriate rural and urban industries, which can be created through applied research, or by adaptation of existing technology to utilise available resources. But technical and managerial skills in the rural and urban labour forces must also be developed, and the tools of the social scientist must be used as a guide to choices of industrialised technology.



These industries will require considerable increases in energy supplies, particularly in rural areas, and hence the development of new sources of energy and new means for energy conservation. The energy problem is also related to the need to increase and diversify agricultural production, to develop forest resources and to improve transportation.

Natural and social scientists must also work together to bring about the social and economic improvements in health that will help to slow down population growth and ultimately stabilise the size of populations.

Throughout the symposium, special emphasis was given to the role of women in the development process, in particular the need for greatly expanding women's opportunities for employ-

ment and education, and for relieving the unallayed drudgery that characterises traditional 'women's work' in rural societies. The unfavourable socioeconomic environment for women would be improved if they could participate more fully in development—the less-developed countries cannot reap the full benefits of technology until this vital human resource has been mobilised. Conversely, the multiple impacts of technology on the welfare of women in changing societies must be evaluated.

Industrialists in both developed and less-developed countries will inevitably have a central role in the application of science and technology to development. To serve their own long-range interests, industries operating in each developing country should be en-

couraged to choose technologies and a scale of operations that are most appropriate for the local factors of production and lead to greater equity within the country, as well as improving the human and natural-resource base.

The participants were unanimous in agreeing that the Singapore symposium should not be an isolated event. A continuing steering committee was established which has begun to explore those problem areas that could be most effectively approached by cooperation among scientists and technologists from the rich and the poor countries. □

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## UNCSTD: gloom over growing gulf between politicians and scientists

"THE gap between the 'experts' and the policy-makers, between those who study and work with science and technology, and those who make development decisions—that gap seems to be growing ever larger as UNCSTD draws near", laments the latest issue of the *Lund Letter*, a Swedish commentary on preparations for the conference.

And a recent Swedish Royal Academy of Sciences meeting has emphasised the point. Called to inform and stir up interest among scientists who may not have been involved in the official preparations for the conference, the day-long discussions left a pessimistic impression of what the official UNCSTD session can hope to achieve.

The non-governmental organisations, however, offer more hope. They came up with some good ideas at their meeting in Singapore in January (see above), and the academy meeting pointed to several areas where Swedish research could be useful to the Third World. The non-governmental organisations seem certain to make a hefty intellectual and psychological contribution.

One suggestion for a new form of cooperation came from Dr Stephan Schwartz, the head librarian at the Royal Institute of Technology, who wanted to improve the flow of scientific and technical information to developing countries. From his own survey of the age distribution of references in scientific journals, it is apparent that in a good journal (*Nature* was his example), half of the references are less than two years old. In a journal used in even a relatively advanced developing country like Portugal, half the references are seven to 10 years old.

This means that the Third World is not getting the most up-to-date information, and is therefore not able to tackle its problems in the most up-to-

date ways. This situation could be improved by establishing link-ups between libraries in developing countries and Western institutes which could do computer searches of the specialised literature needed to cope with the countries' particular problems. Photocopies of most important material could then be supplied to the developing countries.

Dr Schwartz pointed out, however, that the information such a system could supply would be useless unless it could be assimilated by the local science and technology system. He thus echoed one of the meeting's recurrent themes: that development of one sector in a country is only possible if it is backed up by development in all the other sectors. It is these gaps that are hardest to close, because they are the result of development and depend on all other gaps closing first.

The gaps in the politicians' outlooks are so wide as to make them incompatible. In agriculture alone, Asian and African demands are totally different from what the Europeans want to offer.

As Mr Thomas Rosswall from SCOPE (the Scientific Committee on the Problems of the Environment, set up by the International Council of Scientific Unions) pointed out, the European Community (EC) paper presented to UNCSTD's Third Preparatory Committee in January maintains that it is possible, with adequate water and fertiliser supplies, to triple and quadruple yields from traditional varieties of seeds. This is irrelevant to the Third World, where neither adequate water supplies nor fertilisers are available.

In talking about the advantages of fertilisers, the EC paper takes no heed of the fact that widespread use of fertilisers is only possible where their cost, relative to the costs of other agricultural inputs, is low, which is certainly not the case in developing

countries. Again, the EC paper, recounting the European experience, sees mechanisation as a good way of achieving economies of labour. But the Asian and African papers specifically say that they want labour-intensive agricultural systems.

On a more practical note, Dr Bo Hall, of the Department of Agriculture's Commission on Natural Resources, pointed to research on the production of pig iron on a small scale but at prices competitive with the iron produced from big blast furnaces. Small-scale methods are needed for the exploitation of small domestic markets, in contrast to the large-scale technologies developed by the multinationals for developing large ore bodies.

Dr Malin Falkenmark, of the Natural Sciences Research Council's Committee for Hydrology, said that research on groundwater in hard rock is a Swedish speciality and could be useful in India and West Africa especially, where rock groundwater is an important source of water. Groundwater replenishment techniques—techniques for artificially recharging groundwater supplies—were originally developed in Sweden and could probably be used widely in arid and semi-arid areas.

There are vast regions where Swedish work on erosion and sedimentation could also be applied. These processes are not always thought of in relation to river basin development, although many reservoirs rapidly fill up with sand if erosion is not stopped on lands further upstream. Finally, the Nordic countries could pool their work on water assessment—determining the available water resources in an area to avoid over-exploitation—and help developing countries to make water assessments of their own.

Wendy Barnaby

# correspondence

## Jobs: the need to reach an ideal steady state

SIR,—The problem of short-term contract workers is a serious one but it is also very complex and one should beware of facile solutions which can have even more disastrous long term repercussions.

To illustrate the problem one has to look at the ideal steady state. In the biomedical sciences we estimate that there are about 5,000 tenured academic and research posts in United Kingdom Universities and research institutes. There is probably about the same number in the physical sciences. Since each tenured post is occupied for about 35 years the average turnover rate, assuming no wastage, should be about 2.8% per annum. With a uniform age distribution, we could expect 130 vacancies every year in the biomedical sciences simply for renewal. It would mean that we could have a total of 400 people in three year postdoctoral fellowships with excellent chances of their being placed in tenured posts at completion.

The problem is that we are very far from this situation. In 1976, only 20% of the science posts in British universities had holders aged 50 and above, which means that for the next 15 years the retirement rate is going to be about 1% and we can only expect positions to appear about  $\frac{1}{4}$  of that expected from the ideal steady state. Thus the biomedical sciences will only have about 30 to 40 vacancies a year which not only accords more with what we know to be the case, but also tells us that this will be with us to very near the end of the century. The reasons are obvious; this generation is now paying the price for the rapid change in the rate of expansion in the late fifties and sixties; the people they are replacing were appointed before that when the system was much smaller. It is also easy to see that if the step at the below 40 age group is followed by a trough, the long term consequences could be disastrous.

The only solution is to aim to get to the ideal steady state position now. Tenured posts must be offered in universities at the average turnover rate, but in order to do this the system will have to undergo an absolute expansion. Naturally this bulge should not enter into the calculation of the turnover rate, but should be seen only as a borrowing against future vacancies. It seems to me that, in the main, this has to be done largely by government intervention as part of a deliberately planned policy. Nevertheless, there are contributions which might be made by private foundations and possibly by research councils. The best they could do would be to create senior research positions in universities for people already holding university posts. These would all be *ad hominem* appointments so that the subject areas and the candidates are selected by these organisations, and the posts are not simply given away with no control. However, the position vacated by such an appointment can now become available to the university for a new entrant. In essence, in this scheme, the outside organisation is only temporarily

committing itself to a university for the length of tenure of the post, perhaps 15 years; for new tenured entrants, the commitment is for 40 years and only the universities can do that.

From this analysis it can be seen that temporary posts do not help, except as offering some small buffering capacity. They can only be created and used effectively against future vacancies, and unless accompanied by other actions they can only worsen the situation. A prolonged postdoctoral period can be seen as a mechanism which gives the occupants several additional opportunities to apply for permanent posts as they arise annually. This does not help as long as the input further back in the system provides excessive competition, and the difficulties are further compounded by the universities choosing younger candidates for lectureships because they are cheaper than the man with several years experience. We have also to tackle the question of the number of people doing PhD degrees, and this raises a whole new set of problems. What is a PhD degree for? If it is seen as the first step towards a research or academic career, and this is the expectation of people doing it, then we are producing too many, and the numbers should be cut. Universities would strongly oppose that step, because PhD students provide a cheap source of research labour in many university science departments. We have to look at postgraduate education and training as a matter of urgency; it is clearly impossible and undesirable for every university to have a graduate school in every subject, since that puts demands on resources that cannot be met.

The question of the short-term workers is only the tip of the iceberg and the central problems are deepseated and long term. In some way, everybody involved needs to be got together to find a national solution; and unless this is done soon and in a concerted manner I, for one, am very nervous about the long term future for British science and higher education.

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## Little danger from radon

SIR,—Recently, *Nature* reported (30 November 1978, page 431) the work of Berkeley scientists who have expressed concern that reduced ventilation rates may increase the hazard from the inhalation of the short-lived daughters of  $^{222}\text{Rn}$ . This problem has been under consideration by the National Radiological Protection Board in recent years and we think that the conclusions reached by the workers at Berkeley should be approached with caution.

They based their case on extrapolation from uranium miners' medical data. For uranium miners, W. Jacobi (Proceedings of NEA Specialist Meeting, Elliot Lake, Canada, OECD, Paris pp. 33-38; 1977) has estimated 200 excess lung cancer deaths among a population with a cumulative exposure of  $10^6$  Working Level Months. (The Working Level Month

**Table 1** Lung cancer incidence predicted due to environmental radon daughter exposure

Winter ventilation rate $\text{h}^{-1}$	Mean population exposure WLM $\text{y}^{-1}$	Lung cancer cases predicted per $10^6$ population per year
0.8	0.15	30
0.5	0.22	44
0.4	0.28	56
0.3	0.38	76
0.2	0.58	116
0.1	1.15	230

(WLM) is 170 hours exposure at 1 Working Level (WL); the Working Level is a measure of the concentration of the potential alpha energy in air from any combination of the short-lived daughters of radon.  $1 \text{ WL} = 1.3 \times 10^8 \text{ MeV m}^{-3}$ . A recent survey (Cliff, K. D., *Phys. Med. Biol.* 23, 696-711; 1978) estimated that the average population exposure rate domestically to radon daughters for Great Britain is about 0.15 WLM per year.

From the above figures it is possible to deduce, for the female population of Great Britain (assuming a 20 year latency period) that inhalation of radon daughters has caused more bronchus and lung cancer deaths up to the age of 40 than actually occur from every single cause, according to mortality statistics. This result shows the danger of applying a risk estimate derived from a special group to other groups of the population. Even if allowance were made for more rapid room ventilation in the days of open fires, we would still deduce that lung cancer incidence among women under 40 was entirely attributable to radon, and that smoking made little contribution—an untenable conclusion.

Is a WLM to a working miner the same, from a comparative dosimetry point of view, as that to a member of the general population? Studies show that, to within a factor 2, there is no difference in the deposition of radon daughters in the lung. In the absence of any other data, apart from that for miners, on which to base a risk estimate for the general population we may use Jacobi's figure to consider the implications of a general reduction in ventilation rates during winter. We assume a mean ventilation rate in summer (5 months) of two room changes per hour and that energy conservation efforts will not alter this rate. Table 1 shows the predicted incidence of lung cancer in the population (male and female) of Great Britain as the mean winter (7 months) ventilation rate is reduced. The current total lung cancer incidence in Great Britain is about 650 per  $10^6$  population per year.

It cannot be stressed too strongly that such calculations as given in Table 1 should be regarded as speculative and represent an absolute upper limit to possible lung cancer incidence attributable to environmental radon daughter concentrations.

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# news and views

## Clinical relevance of opiate receptor and opioid peptide research

from Solomon H. Snyder

OPIATE receptors<sup>1</sup> and the enkephalins, their endogenous peptide ligands<sup>2</sup>, have advanced the understanding of numerous brain functions and provided several potential clinical applications.

The localisation of opiate receptors as revealed in the elegant autoradiographic studies of M. J. Kuhar<sup>3</sup> has clarified the neuronal sites at which these drugs exert their pharmacological effects. Opiate overdose causes death by depressing respiration. High densities of opiate receptors in the nucleus of the solitary tract, a respiratory reflex centre, suggest likely sites of this activity. Opiates constrict the pupils, accounting for the 'pinpoint' pupils of heroin addicts, an effect apparently attributable to opiate receptors in the pretectal nuclei. Opiate receptor concentrations within the limbic system may mediate euphoric effects. If opiate receptors vary in drug specificity throughout the brain, as suggested by pharmacological data<sup>4</sup>, novel drugs acting selectively on individual receptor populations might afford selective therapeutic advantage.

### Opiate addiction

The opiate receptors which are highly concentrated in the locus coeruleus may have direct relevance for treating opiate addicts, especially their withdrawal symptoms. Though the locus coeruleus contains only 1,400 neuronal cells, all contain noradrenaline and their axons project ubiquitously, providing the major adrenergic innervation of the central nervous system. Micro-injections of opiates selectively inhibit firing of locus coeruleus neurones but fail to alter the activity of closely adjacent cell groups which do not possess opiate receptors<sup>5</sup>. Besides opiate receptors, locus coeruleus cells also possess  $\alpha$ -adrenergic autoreceptors, whose stimulation by noradrenaline or clonidine, which mimics noradrenaline at  $\alpha_2$ -receptors slows their firing<sup>6</sup>. Mor-

phine tolerance and physical dependence in rats is accompanied by tolerance of the locus coeruleus to the slowing effects of opiates but not of clonidine<sup>6</sup>. Opiate withdrawal in these rats elicits extremely rapid firing of locus coeruleus neurones.

Drugs which block the  $\alpha_2$ -adrenergic receptors, such as piperoxane and yohimbine, accelerate the firing of locus coeruleus neurones and produce subjective effects of anxiety and agitation as well as hypertension in humans. As these symptoms resemble opiate withdrawal, some abstinence symptoms might result from enhanced locus coeruleus activity. If this were the case, drugs slowing locus coeruleus neuronal firing might alleviate opiate withdrawal symptoms. Gold *et al.*<sup>7</sup> observed a dramatic alleviation of abstinence symptoms in human methadone addicts treated with clonidine. Thus, clonidine or related agents may prove useful treatments for opiate withdrawal. Conceivably, maintenance therapy with such drugs would ease the craving of addicts for heroin.

### Developing new analgesics

Simple test tube assays to measure opiate receptor binding have greatly facilitated the screening of potential new opiate-related drugs. More important, the discovery that low concentrations of sodium ion selectively differentiate the actions of agonists, antagonists and mixed agonist-antagonists at opiate receptors may facilitate development of potentially less addictive opiates<sup>8</sup>. Sodium 'weakens' the binding of pure agonists to opiate receptors by 15–100-fold, while not influencing the binding of pure antagonists at all. Drugs with both agonist and antagonist activity are affected in an intermediate fashion. Before biochemical labelling of the opiate receptor these mixed agonist-antagonist analgesics, which have reduced addictive potential, were difficult to detect by tests in intact animals. Opiate receptor screening has simplified their identification. Until recently, pentazocine (Talwin) was the only mixed agonist-antagonist opiate available

commercially. Though less addictive than conventional opiate agonists, pentazocine does not relieve severe pain as well as does morphine. At therapeutic doses pentazocine sometimes elicits anxiety, depersonalisation and psychotomimetic effects. A 'second generation' of mixed agonist-antagonist opiates are now appearing on the market. Butorphanol (Stadol), from the same benzomorphan class as pentazocine, is more potent and might conceivably have fewer psychotomimetic effects. Nalbuphine and buprenorphine are very promising mixed agonist-antagonists which may have even greater potential, but are not yet widely enough used to judge their liability to cause addiction.

### Enkephalin analogues

Numerous pharmaceutical concerns have developed enkephalin analogues for possible therapeutic uses, hoping that derivatives of such a natural substance might be non-addicting. The amino acid sequence of Met-enkephalin is tyrosine-glycine-glycine-phenylalanine-methionine, while Leu-enkephalin differs from Met-enkephalin simply by the substitution of a carboxyl-terminal leucine for methionine. Replacement of the glycine at position 2 by D-alanine greatly reduces the proteolytic susceptibility of enkephalin. Other modifications increase receptor affinity, facilitate penetration of the blood-brain barrier, and enhance absorption from the gastrointestinal tract. The Sandoz Company reported a pentapeptide FK-33824 which differs from Met-enkephalin only in the replacement of glycine-2 with D-alanine, the N-methylation of phenylalanine and alteration of the methionine by oxidising its sulphur to a sulfoxide and conversion of the carboxyl to a carbinol<sup>9</sup>. This drug is 30,000 times more potent than enkephalin when injected into the brain, is an active analgesic when administered parenterally and orally and has already been evaluated in humans<sup>10</sup>. The facial flushing produced by FK-33824 resembles the naloxone-reversible facial flushing in

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some diabetics given the anti-diabetic drug chlorpropamide followed by alcohol, implicating enkephalin in the pathophysiology of certain types of diabetes<sup>11</sup>. In some animal tests, FK-33824 elicits physical dependence, but is less 'addictive' relative to its analgesic activity than are conventional opiate agonists (D. Romer, personal communication). A possible successor to FK-33824 has already been developed simply by adding an N-methyl group to the tyrosine<sup>12</sup>. The resultant methyl-FK-33824 is five times more potent than FK-33824 by subcutaneous injection and orally. Its oral form is equipotent with morphine.

The Lilly Pharmaceutical Company has developed an enkephalin analogue differing from 2-D-alanine-Met-enkephalin simply by methylation of methionine's amino nitrogen and conversion of methionine to an amide. This agent, LY-127623 seems to have similar potency to FK-33824 (refs 13, 14) when administered parenterally but is less active when given by mouth.

LY-127623 may be less addictive. Withdrawal symptoms in morphine-addicted rats are much less severe with LY-127623 than with other conventional opiate agonists or mixed agonist-antagonists examined. Also, at equi-analgesic doses LY-127623 produces less respiratory depression than morphine. In numerous pharmacological tests LY-127623 and FK-33824 behave

like pure opiate agonists. Thus these enkephalin analogues may be unique as agents outside the mixed agonist-antagonist group with reduced propensity to cause addiction. The absence of direct comparisons between LY-127623, FK-33824 and other enkephalin analogues precludes conclusions about their comparative benefits.

Drugs which inhibit enzymes that inactivate endogenous substances are often powerful therapeutic agents—for example, acetylcholinesterase and monoamine oxidase inhibitors. Enkephalin can be degraded by a wide range of nonspecific peptidases. Recently Malfroy *et al.*<sup>15</sup> identified a specific 'enkephalinase' in brain membranes. This enzyme has a much greater affinity for enkephalin than for most other peptides. Its regional distribution throughout the brain resembles that of the opiate receptor. Moreover, in rats chronically treated with morphine, enkephalinase activity increases substantially, while other peptidases show no change. Drugs which facilitate enkephalin action by blocking this enzyme might have unique applications extending beyond the opiate field. Enkephalinase seems closely similar to the angiotensin-converting enzyme which transforms the inactive angiotensin I to the physiologically active angiotensin II<sup>16</sup>. This same enzyme also inactivates bradykinin, a peptide which regulates inflammation, vascular shock and pain perception. Bradykinin has recently been localised to specific neuronal systems in the brain<sup>17</sup>. Thus, enkephalinase inhibitors might influence bradykinin and angiotensin as well as

enkephalin.

A role for opioid peptides in mental illness was suggested by preliminary reports of a beneficial effect of the pituitary peptide  $\beta$ -endorphin in schizophrenic and depressed patients<sup>18</sup> and reduced auditory hallucinations in schizophrenics treated with naloxone<sup>19</sup>. These findings emerged from uncontrolled studies and await full confirmation in double-blind placebo-controlled experimental designs. Support for effects of opioid peptides in schizophrenia emerges from the positive influence of FK-33824 on hallucinations and overall mental state in a study of eight patients in Denmark<sup>20</sup> and in six schizophrenics in Munich (N. Nedopil & E. Ruther, personal communication). In the Danish study patients received intramuscular injection of 1, 2 and 3 mg on three successive days, while in the German study 0.5 mg and 1.0 mg were given in intravenous infusions on two successive days. Unfortunately, neither of these studies was conducted in a double-blind fashion.

One controlled study suggests beneficial effects in schizophrenia of an opioid-related peptide which has no opiate activity. [Des-Tyr<sup>1</sup>]- $\gamma$ -endorphin comprises amino acids 62–77 of the pituitary peptide,  $\beta$ -lipotropin. The absence of an N-terminal tyrosine abolishes affinity for opiate receptors. On the basis of its behavioural effects in rats<sup>21</sup> [des-Tyr<sup>1</sup>]- $\gamma$ -endorphin was administered to chronic schizophrenics<sup>22</sup>. In a double-blind placebo-controlled crossover design the peptide in daily doses of only 1 mg dramatically alleviated schizophrenic symptoms. □

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## Prostacyclin in blood vessel-platelet interactions: perspectives and questions

from Philip Needleman

BLOOD vessels possess the capacity for the intrinsic synthesis of vasodilator prostaglandins, thereby providing considerable potential for the local regulation of vascular tone<sup>1</sup>. Prostacyclin<sup>2,3</sup> or its stable metabolite 6-keto-PGF<sub>1 $\alpha$</sub> <sup>4,5</sup> is the primary vascular prostaglandin produced. As prostacyclin (PGI<sub>2</sub>) is a potent inhibitor of platelet aggregation *in vitro*<sup>6</sup> it thereby became a prime candidate for the local vascular regulation of thrombotic events.

Isolated blood vessel preparations rather inefficiently (<5%) convert exogenous arachidonate (by way of the enzyme cyclooxygenase) into prostacyclin.

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glandin endoperoxides. However, the prostacyclin synthetase in vascular tissue quantitatively (>80%) metabolises exogenous or endogenous PG endoperoxides into PGI<sub>2</sub>. On the other hand, platelets possess a very active cyclooxygenase which readily cyclises arachidonate into endoperoxide which is then enzymatically converted into the potent aggregator and blood vessel constrictor, thromboxane A<sub>2</sub>. Bunting *et al.*<sup>6</sup> and Moncada *et al.*<sup>7</sup> found that blood vessel segments treated with indomethacin (a reversible cyclooxygenase inhibitor) and stirred with platelet-rich plasma, prevented aggregation in mixing experiments. They therefore proposed the hypothesis that endoperoxides are released from platelets during aggregation and are used by

the blood vessels to synthesise prostacyclin. Such an exchange of products simultaneously bypasses the inefficient vascular cyclooxygenase and deprives the platelets of substrate for thromboxane synthesis. An implication of such an interaction is that thrombosis and vascular tone may be mediated or modulated by the local balance of the ratio of vascular-prostacyclin/platelet-thromboxane.

An understanding of platelet and blood vessel arachidonate metabolism and interactions has therefore become the focus of considerable research. In this issue of *Nature* (page 66), Hornstra, Haddeman and Don report data which demonstrate that unstimulated or collagen-activated platelets do not seem to release endoperoxides which could be used as substrate for PGI<sub>2</sub> synthesis by vascular fragments. They also observe that indomethacin inhibition of the aortic cyclooxygenase was reversible, and that intrinsic vascular PGI<sub>2</sub> was the culprit that inhibited the platelets in mixing experiments. We recently demonstrated<sup>8</sup> that exogenous [<sup>14</sup>C]-arachidonate added to intact human platelets in the presence of blood vessel microsomes (as a source of prostacyclin synthetase), resulted in the production of [<sup>14</sup>C]-thromboxane B<sub>2</sub> only. PG endoperoxides were released from the intact platelets only when thromboxane synthesis was inhibited by imidazole (an antagonist of thromboxane synthetase *in vitro*); the blood vessel microsomes converted the released endoperoxide into PGI<sub>2</sub>. Furthermore, incubation of unstimulated or thrombin-activated human platelets which contain [<sup>14</sup>C]-arachidonate-labelled phospholipids, with aspirin-treated intact aorta, immediately resulted in adhesion. The only cyclised arachidonate product of this platelet-blood vessel adhesion reaction was labelled thromboxane, while no labelled 6-keto-PGF<sub>1α</sub> was detectable. However, thrombin stimulation of imidazole-inhibited platelets resulted in the release of platelet-derived labelled PG endoperoxides which were converted to labelled PGI<sub>2</sub> by the vascular prostacyclin synthetase. Comparable results have been obtained by Baenziger *et al.*<sup>9</sup> (*Cell* in the press) who incubated cultured human arterial smooth muscle

and venous endothelial cells with human platelets in the presence of arachidonate and measured PGI<sub>2</sub> production by bioassay (that is, inhibition of [<sup>14</sup>C]-serotonin release from platelets). Significant prostacyclin synthesis from platelet-derived PG endoperoxides by cultured cells pretreated with aspirin was observed only when platelet thromboxane synthesis was inhibited. All these results mitigate against the attractive notion that blood vessels derive endoperoxide from platelets for subsequent conversion to PGI<sub>2</sub> and are thereby protected from deposition of platelets on vessel walls. Fortunately, however, these experiments have unmasked a pharmacological strategy which may enable one to manipulate the platelet-blood vessel interaction to improve the local prostacyclin/thromboxane ratio. This might be useful therapeutically in thrombosis, coronary and cerebral vasospasm and perhaps atherosclerosis. Development of a thromboxane synthetase inhibitor effective *in vivo* could facilitate the release of platelet endoperoxide on which vascular prostacyclin synthetase could then act. As vascular injury initiates platelet adhesion and aggregation, a thromboxane synthetase inhibitor has the potential selectively to generate prostacyclin right at the critical site.

Despite the demonstration of a fascinating range of pharmacological effects, the physiological and pathological role of vascular prostacyclin production and its effect on platelet function still needs considerable clarification. For example, it was initially suggested that vascular endothelial cells were the primary vascular source of the anti-thrombotic PGI<sub>2</sub>, and loss of endothelium during injury, therefore, favoured local thrombosis<sup>10</sup>. However, subendothelial vascular smooth muscle readily produces prostacyclin under conditions which favour thrombosis<sup>11</sup>. It is difficult to reconcile the synthesis of PGI<sub>2</sub> by blood vessels with damaged endothelium with the idea that PGI<sub>2</sub> is one of the chief deterrents of thrombosis.

Another thought-provoking platelet-blood vessel interaction was recently reported in *Nature* by Gryglewski *et al.*<sup>12</sup> and Moncada *et al.*<sup>13</sup> Their discovery suggests that prostacyclin might be continuously synthesised by the lungs and released as a circulating hormone into the arterial blood. However, the actual PGI<sub>2</sub> levels present in the arterial blood must be ascertained in more physiological circumstances. In the above studies the demonstration of arterial PGI<sub>2</sub> levels was in anaesthetised heparinised animals in which the blood was recirculated through a peristaltic pump. By analogy, previous experience with intact animals has demonstrated that high levels of renal pro-

staglandins are produced in anaesthetised, surgically prepared dogs, whereas little if any renal PG production occurs in unanaesthetised trained animals. Indeed, if PGI<sub>2</sub> is circulating in high enough concentrations to interfere with platelet aggregation, then platelet cyclic nucleotide concentrations should be raised in freshly prepared platelet-rich plasma, which is not the case. Furthermore, it should be possible to demonstrate circulating levels of 6-keto-PGF<sub>1α</sub> (by immunoassay and mass spectrometry for example) in blood drawn from anaesthetised experimental animals or man. The resolution of some of these intriguing questions may help to clarify the physiological and pathological role of intrinsic prostacyclin synthesis in regulating platelet function. □

## Iron storage in bacteria

from Pauline M. Harrison

ENTERIC and other bacteria have been known for some time to synthesise low molecular weight siderophore molecules of high iron affinity, which enable them to scavenge the iron they require from the medium, even when it is present in low concentration. The bacteriostatic effects of the iron-carrier in serum, transferrin, and of similar molecules in milk and in egg-white may be due to competition by these molecules with the siderophores for iron. Synthesis of siderophores is regulated by negative feedback mechanisms, which shut off their synthesis when iron is abundant. The need for a means of storing iron in bacteria has not been apparent and until recently no counterpart has been found of the iron-storage molecule, ferritin, which occurs in a variety of eukaryotic species from fungi to man. Now ferritin, or a ferritin-like molecule, has been found in several bacterial species. The molecule characterised by Stiefel and Watt (this issue of *Nature* page 81) as a bacterioferritin was first isolated from the nitrogen-fixing bacterium *Azotobacter vinelandii* by Bulen and coworkers (*Biochem. biophys. Res. Commun.* **54**, 1274; 1973) who described the molecule as a *b*-type cytochrome containing large amounts of non-haem iron and no labile sulphide. Stiefel and Watt now show that this molecule is not only a haem protein, but closely resembles mammalian ferritin in several respects. The combination of both types of iron within a single molecule is most unusual and is not known in ferritins

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13. Moncada *et al.* *Nature* **273**, 767 (1978).

of higher organisms. To what extent then are Stiefel and Watt justified in designating their molecule a bacterioferritin?

Ferritin from animal or plant sources is a molecule of potentially high but variable iron content (up to at least 30% by weight or over 4,000 Fe atoms per molecule) held within a protein shell. Its iron is in the form of 'micelles' or microcrystals of hydrous-ferric-oxide-phosphate, which can be seen in the electron microscope without staining or shadowing as particles of about 60 Å diameter. This property of ferritin enables it to be recognised within cells and it has been used as a marker for other molecules tagged to it. It has been shown in several species that iron stimulates the biosynthesis of ferritin: protein subunits assemble to form shells, which accumulate iron. A recent high resolution electron density map of horse spleen apoferritin (Banyard, Stammers & Harrison *Nature* **271**, 282; 1978) shows the molecule as a symmetrical assemblage of 24 subunits each of 18,500 molecular weight with high helix content giving an outer diameter of 130 Å. The structures of several other crystalline ferritins are very similar, but some animal and plant ferritins may be larger. Mössbauer spectroscopy of horse spleen ferritin provides evidence of anti-ferromagnetic ordering within the iron cores at low temperatures, the ordering temperature depending on iron content and particle size.

The 'bacterioferritin-cytochrome' of Stiefel and Watt has an iron content of 13–20% by weight, a subunit weight of 17,000, a molecular diameter comparable to that of animal ferritin and an electron-dense core of 55 Å. Its magnetic susceptibility and temperature-dependent Mössbauer spectra establish it as containing weakly-coupled high-spin  $\text{Fe}^{\text{III}}$  reminiscent of ferritin iron. However, the presence of protoporphyrin IX (one per two protein subunits) is distinctive and the potential required to reduce both forms of iron is more negative than that for horse spleen ferritin. Moreover, the bulk of the  $\text{Fe}^{\text{II}}$  is retained inside the molecule on gel filtration, whereas it is more readily lost from the horse spleen protein, presumably diffusing out of the molecule through channels in the protein shell seen in the electron density maps. Stiefel and Watt have considered the possibility that their 'bacterioferritin' may act as an electron store or as a specific iron-storage depot for the iron-containing

protein nitrogenase.

At a recent meeting on Proteins of Iron Metabolism\* Bauminger, Cohen, Levy, Ofer and Yariv of the Hebrew University, Jerusalem, described evidence for the presence of a new type of iron-storage compound in *E. coli* grown on  $^{57}\text{Fe}$ -enriched media. Mössbauer spectra of packed cells showed the presence of high spin  $\text{Fe}^{\text{III}}$  with evidence for magnetic ordering below 2.6 K. An iron-rich protein, of approximately 300,000 molecular weight containing electron-dense particles was isolated and gave Mössbauer spectra similar to those of the whole cells, but this soluble protein accounted for only about 1% of the iron content of the whole cells grown on the most iron. These workers consider the pure protein from *E. coli* to be quite distinct from ferritin. They report similar  $^{57}\text{Fe}$  spectra from *Proteus mirabilis* and *Mycoplasma capricolum*. This suggests that the requirement for an iron-storage molecule may be universal.

A similar molecule may also be present as a minor component of a freshwater magnetotactic spirillum in which most of the iron (1.5% of the cell dry weight) is present in chains of magnetite crystals (each about 100 nm across), which account for the alignment of these organisms in the geomagnetic field (Frankel, Blakemore & Wolfe *Science* **203**, 1355; 1979).

It is not yet clear how similar the *E. coli* protein is to that from *Azotobacter vinelandii*, although the chromophore in addition to the non-haem iron also seems to be present in the *E. coli* protein. Fortunately both proteins have been crystallised and the results of further structural studies are eagerly awaited as well as those of biochemical studies which may more clearly establish their biological roles and their resemblance to and differences from ferritin. □

\*Held at Davos, Switzerland on 17–21 April, 1979.

## Errata

In the article 'Acetylcholine receptor clusters' (*News & Views*, **278**, 599; 1979) a line was omitted from the second paragraph, third column, page 599. The sentence beginning on line 12 of that paragraph should read "After the initial stage of cluster formation, the appearance of new hot spots in the absence of a nerve fibre has not been observed on morphologically stable muscle cells."

In the footnote to the article 'Membranes and Parasites' (*News & Views* **277**, 12; 1979) it should be noted that the Special Programme for Research and Training in Tropical Diseases is sponsored by The World Bank, The United Nations Development Programme and the WHO.

## Actinide magnetism: an extraordinary tale

from Gillian Gehring

SOME intriguing experiments on the magnetic properties of actinide intermetallics containing uranium have produced results which cannot be explained by any of the standard theories of magnetism. The most anomalous material is USB (uranium antimonide) which has been extensively studied by Lander *et al.* (Lander *et al. Phys. Rev. B* **14**, 5035; 1976; Lander *et al. Phys. Rev. Lett.* **40**, 523; 1978; Lander *et al. Phys. Rev. Lett.* **42**, 260; 1979) but the behaviour of UN (uranium nitride) which has been studied by Buyers *et al.* (*Proc. Int. Symp. on Neutron Inelastic Scattering*, Vienna, 1978) is similar. However, all the materials  $\text{AnX}$  where An is uranium or neptunium and X is nitrogen, phosphorus, arsenic, antimony, sulphur, selenium or tellurium have the same or similar magnetic phases (Aldred & Lam, *The Actinides: Electronic Structure and Related Properties I* (eds Freeman & Darby) Academic Press, New York, 1974) so that it is possible that what has been seen in USB and UN is actually characteristic of a whole class of compounds and not just due to some freakish property. The properties of a magnetic material depend on a delicate balance between various interactions and it may be that the particular combination characteristic of the actinides; large spin orbit interaction, variable valance and an apparent tendency for metallic f electrons will call for a totally new theory.

Studies of the magnetic properties of materials have been of great importance in helping our detailed understanding of solid state phenomena. This has been, I think, for two main reasons. The magnetic interactions which are odd under time reversal, split electron degeneracies which cannot be removed by any other means so their explanation is a very critical test of our understanding of a material. Also a very wide variety of experiments may be done. Above the phase transition one can measure the susceptibilities of the system to uniform or varying magnetic fields; close to the transition one sees very pronounced fluctuations in the magnetisation which may exist over large regions of space (that is, involving many crystal sites) and decay only slowly with time; at low temperatures one may study the ordered state and measure the magnetic moment per site as a function of

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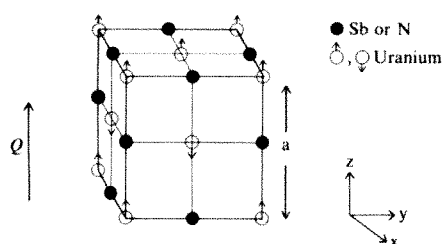


Fig. 1 The magnetic structure of USb and UN for a domain with  $Q = \frac{2\pi}{a}[001]$

temperature and also measure the magnetic excitation energies.

The AnX crystals have cubic, NaCl structure; in Fig. 1 the magnetic structure for USb and UN is shown. The spins are arranged in sheets perpendicular to one of the crystal axes ( $z$  in Fig. 1) so that all the spins in a given sheet are parallel and adjacent sheets are antiparallel; the magnetic moments are perpendicular to these planes. The moment configuration may be described by

$$m_n = zme^{iQ \cdot R_n} \quad Q = \frac{2\pi}{a}[001]$$

where  $R_n$  is the position vector of the uranium site. In an antiferromagnetic crystal it is very useful to specify the  $Q$  vector which characterises the order. Above the phase transition the  $x$ ,  $y$  and  $z$  directions are all equivalent so there are three possible  $Q$  vectors corresponding to ferromagnetic planes perpendicular to the  $x$ ,  $y$  or  $z$  direction. However, once the planes have been chosen the spins are constrained to lie parallel to  $Q$ . The magnetic moment on the uranium site is  $2.82 \mu_B$  for USb; this is comparable with that of the free ion  $U^{3+}$  ( $J=9/2$ )  $3.42 \mu_B$  or  $U^{4+}$  ( $J=4$ ) of  $3.34 \mu_B$  (Lander *et al. op. cit.* 1976). The magnetic moment distribution on the U sites has been shown to be an oblate spheroid: that is, flattened in the  $z$  direction. The transition temperature of USb is 241.2 K which is considerably higher than that of the equivalent lanthanide compound NdSb for which  $T_N = 13.6$  K.

The high temperature and critical behaviour of USb and UN have been studied by neutron scattering by Lander *et al.* and Buyers *et al.* Since the ordering which is going to occur at low temperatures is characterised by  $Q$  then the system will be expected to show a diverging susceptibility to a magnetic field which has the same alternating spatial dependence, that is

$$h_n = z h_0 e^{iQ \cdot R_n}$$

Such a spatially varying field may be provided by scattering a neutron through wave vector  $Q$ . In such an experiment one looks only at one domain because the scattering done at

or near  $Q = \frac{2\pi}{a}[001]$  for example, will

arise only from those parts of the crystal which are ordering with ferromagnetic planes perpendicular to  $z$ . Above the phase transition the clusters of spins which are ordered are small and they grow as the transition is approached. In both USb and UN the aligned clusters are predominantly in the planes; the correlation length is five times larger in the plane as out of it for USb, for UN it is a factor of three. This is not unusual in layer compounds but USb is cubic so that the neighbouring uranium sites out of the plane are at the same distance as those in the plane. (A very small distortion of less than 0.1% does actually occur at low temperatures.) In addition the direction of the spins is also rigidly fixed perpendicular to the planes so that the system has a negligibly small response to a magnetic field which is perpendicular to  $Q$ . Systems in which the moment may only point up or down are known as Ising systems; they are characterised by a slow relaxation time above  $T_N$ , as is found in USb and UN, and their magnetisation tends to zero at  $T_N$  like  $(T_N - T)^{0.3125}$  (in USb the exponent was found to be  $0.32 \pm 0.02$ ). Again this highly anisotropic behaviour has not been observed before in a cubic compound.

The excitation spectrum at low temperatures is even more anomalous. In ordered magnets the elementary excitations are spin waves. These have been seen in systems, such as  $MnF_2$  or  $NdSb$ , in which the electrons which are responsible for the moment are localised on the magnetic site, and in itinerant ferromagnets such as metallic nickel. The form of the excitation and the way in which its energy depends on wavelength are sketched in Fig. 2 for two extreme models. In the Ising model the angular momentum component in the  $z$  direction on one site has been reduced by one unit. Although this is a localised excitation we may consider a linear combination of these states with wave vector  $k$  but the energy will be independent of  $k$ . This situation arises if the angular momenta are constrained so that only the  $z$  component may be non-zero. The opposite extreme is the Heisenberg model. In

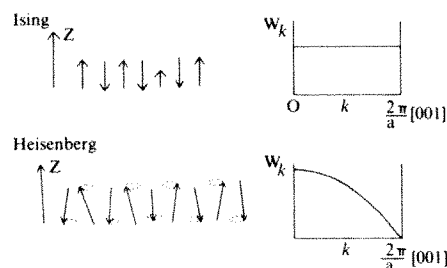


Fig. 2 Sketch of the form of the excitations in Ising and Heisenberg systems with the type of dispersion relation— $w_k$  versus  $k$  plot.

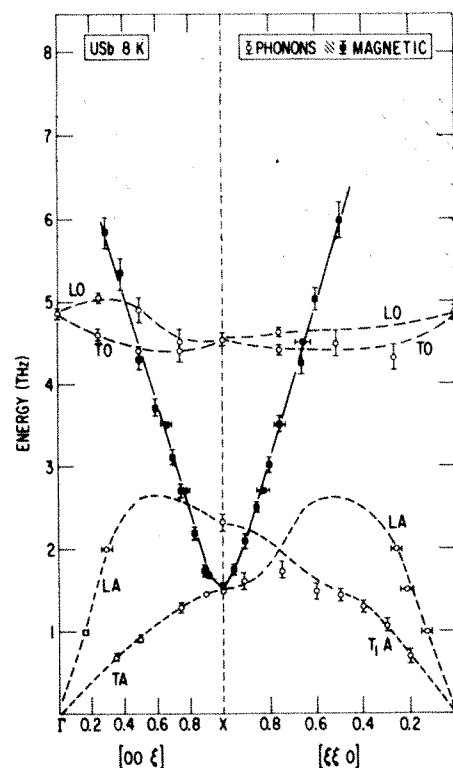


Fig. 3 The dispersion curves for USb; energy plotted against wave-vector transfer  $Q$  (in units of  $2\pi/a$ ). The dashed lines represent the phonon dispersion and are based on the measured open points as well as on our knowledge of phonons in NaCl structures. The magnetic modes are represented by solid squares (the collective excitation) and the hatched area (excitonic level). (From Lander *et al. Phys. Rev. Lett. op. cit.* 1979).

this case all the components of angular momenta may be allowed to be non-zero and an exchange coupling of the form  $\mathcal{J}(\mathbf{r}_{nm})(J_n^x J_m^x + J_n^y J_m^y)$  can transfer the excitation from site  $n$  to  $m$ . The energies of the excitations vary strongly with  $k$ . An intermediate case will arise if there is some anisotropy energy favouring the  $z$  direction and the excitation spectrum will show some dispersion. As the temperature is raised many moment deviations occur; the effect of this is that the energy of the excitations fall (approximately with the magnetisation) and they become less well defined as they scatter off each other. These excitations may be created by a transverse time-dependent magnetic field: this is because the operators which take state  $|J, M_J\rangle$  into  $|J, M_J \pm 1\rangle$  are  $J^x \pm iJ^y$ . At low temperatures a time-dependent magnetic field in the  $z$  direction has very little effect because it cannot create these spin waves. Near to  $T_N$  there is a diffusive response to a time-dependent field  $h_z$ . This behaviour of the transverse and longitudinal excitations has been observed in essentially all ionic and metallic magnets.

The behaviour in USb and UN is quite different. Neither crystal shows



any response to a transverse magnetic field. In USb there is a well-defined excitation for  $T < T_{N/2}$  which has a lot of dispersion as shown in Fig. 3. Its energy increases slightly as the temperature is raised, it is characteristic of a system with equal strengths of interactions between sites in and perpendicular to the planes, and it is excited only by a field in the z direction. In UN there is some evidence of a similar excitation but it becomes very strongly damped away from the point at which it has its minimum energy. Incidentally the minimum energy of this

excitation appears to coincide exactly with the zone boundary energy of the transverse acoustic phonons in both compounds.

There is no way in which the well known theories of localised magnetism nor the theories of itinerant magnetism which work for the transition metal elements and alloys can account for these features. Something really new will be needed in which the interesting but difficult question of localised *versus* itinerant description of electrons will be raised again but this time including spin orbit coupling as a large effect. □

## Bacterial chemotaxis and protein carboxymethylation

from Gerald L. Hazelbauer

MOTILE bacteria migrate along chemical gradients toward higher concentrations of some compounds (attractants) and away from higher concentrations of others (repellents). A number of recent studies of the biochemistry of chemotactic behaviour in *Escherichia coli* and *Salmonella typhimurium* document the central importance of carboxymethylation of a group of membrane proteins. This constitutes significant progress in the understanding of the bacterial sensory response system and is particularly tantalising since protein carboxymethylation has been detected in a large number of mammalian tissues (Kim *et al.* *J. Neurochem.* **24**, 625; 1975; Diliberto & Axelrod *J. Neurochem.* **26**, 1159; 1976) and at least in some cases the reaction appears to be correlated with cellular response to chemical stimuli (Diliberto *et al.* *Proc. natn. Acad. Sci. U.S.A.* **73**, 4050; 1976; O'Dea *et al.* *Nature* **272**, 462; 1978).

An *E. coli* cell swims in straight lines punctuated every few seconds by episodes of uncoordination, called tumbles, that result in new, randomly-chosen directions of swimming. A change in concentration of an active compound over either distance (spatial gradient) or time (temporal gradient) results in an alteration in tumble frequency. Favourable changes suppress tumbles; unfavourable ones induce them. Thus a cell makes net progress along a spatial gradient by longer path lengths in favourable directions. Coordinated swimming occurs when the left-handed helices of bacterial flagella rotate counterclockwise and tumbling occurs when they rotate clockwise. The sensory system in-

fluences migration by controlling the direction of flagellar rotation and the probability of reversals. Sensitivity to active compounds is mediated by specific receptors, some of which have been identified as particular cell surface proteins.

The bacterial response to a gradient is transient. Addition of an attractant to a cell suspension results in an immediate suppression of all tumbles and addition of repellent induces continual tumbling. However after a time ranging from seconds to several minutes, depending on the compound and the magnitude of the gradient, the cells resume their initial behavioural pattern of swimming and tumbling even though the attractant or repellent is still present. Thus bacteria, like many sensory cells in higher organisms, adapt. An important body of work primarily by Goy, Springer and Adler establishes a convincing correlation between adaptation and methylation or demethylation of the methylaccepting chemotaxis proteins (MCPs), polypeptides of approximately 60,000 molecular weight that reside in the bacterial cytoplasmic membrane (see Goy & Springer in *Taxis and Behavior* (Ed. Hazelbauer) Chapman and Hall, 1978 for a summary of that work).

MCPs are methylated by donation of methyl groups from S-adenosylmethionine to form glutamyl 5-methylesters (Kleene *et al.* *J. biol. Chem.* **252**, 3214; 1977; Van Der Werf & Koshland *J. biol. Chem.* **252**, 2793; 1977). The reaction is catalysed by a soluble carboxymethyltransferase apparently specific for MCPs (Springer & Koshland *Proc. natn. Acad. Sci. U.S.A.* **74**, 533; 1977). Mutations in *che* genes generally perturb tactic behaviour. In both *E. coli* and *S. typhimurium* one *che* gene appears to produce the methyl transferase and another a demethylase

(Stock & Koshland *Proc. natn. Acad. Sci. U.S.A.* **75**, 3659; 1978).

Goy *et al.* *Proc. natn. Acad. Sci. U.S.A.* **74**, 4964; 1977) measured the levels of methylation of MCPs during tactic behaviour by *E. coli*. Unstimulated cells exhibit a basal level of methylation. On stimulation the extent of methylation increases (for favourable changes) or decreases (for unfavourable changes), over a period that corresponds to the adaptation time, to a new level which is maintained as long as the chemical environment remains unchanged. The new level of methylation is a function of the magnitude of the stimulus. The straightforward interpretation of these observations is that adaptation is the result of methylation. That predicts that cells unable to methylate MCPs should be unable to adapt. Springer *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **74**, 183; 1977) had previously shown that methionine-starved cells, which should lack the S-adenosylmethionine necessary for methylation, do not adapt but that addition of exogenous methionine subsequent to tactic stimulation allows a delayed adaptation to proceed. Recent studies of *cheX* mutants of *E. coli* by Parkinson and Revello (*Cell* **15**, 1221; 1978) and Goy *et al.* (*Cell* **15**, 1231; 1978) demonstrate that cells in which methylation of MCPs is defective as the result of a mutational block are also defective in adaptation. *CheX* mutants exhibit low levels and aberrant patterns of MCP methylation in both unstimulated and stimulated cells, a phenotype consistent with the *cheX* product being the methyltransferase (Stock & Koshland *op. cit.*). In a constant environment the mutants almost never tumble but temporal gradients of repellents induce tumbling. Using cells induced to tumble, both laboratories showed that temporal gradients of attractants suppress tumbles. The striking defect in the mutants is that they exhibit greatly extended adaptation times. In fact *cheX* cells subjected to relatively strong stimuli maintain the stimulus-induced behaviour of continual tumbling or exclusively smooth swimming for as long as the investigator cares to wait (36 h in some cases). It appears that *cheX* mutations do not significantly affect the ability of cells to be excited by tactic stimuli but only the ability to adapt to them. As the authors suggest it seems likely that the smooth-swimming phenotype of *cheX* strains reflects an inability to adapt to previously encountered stimuli rather than a defect in the tumbling mechanism itself. In any case the observations that two independent blocks on methylation of MCPs result in the same adaptation-defective phenotype provides strong evidence that the reaction is central to

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adaptation.

In addition it is clear that the MCPs are critically important for the transduction of tactic signals from receptors to the flagellar motor. Springer *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **74**, 3312; 1977) and Silverman and Simon (*Proc. natn. Acad. Sci. U.S.A.* 3317; 1977) both demonstrated that two different genes, *tsr* and *tar*, code for MCPs. Mutations in *tsr* eliminate response to one set of stimuli (serine, some other amino acids and some repellents) and those in *tar* to a complementary set (aspartate, maltose and some repellents). Thus excitation mediated by one group of receptors requires the *tsr* function and by another group requires *tar*. The division extends to the methylation reaction. *tsr*-mediated stimuli result primarily in methylation of MCP I, the product of *tsr*, and *tar*-mediated stimuli cause methylation of MCP II. Both sets of authors suggest that MCPs I and II together accept tactic signals from most if not all receptors and thus represent the final stage of signal transduction.

That suggestion may require some revision since Kondoh *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **76**, 260; 1979) describe an MCP III which is methylated upon stimulation by galactose,

ribose or their analogues and is independent of *tsr* and *tar*. This is particularly interesting since the acceptor of signals from galactose and ribose receptors is thought to be the product of the *trg* gene. Mutants in *trg* do not respond to gradients of ribose or galactose but respond normally to other attractants (Ordal & Adler *J. Bact.* **117**, 517; 1974; Hazelbauer & Harayama, *Cell* **16**, 617; 1979). MCP III is not methylated in *trg* mutants but it is not yet clear if the *trg* product is the MCP III protein or instead is a first-stage transducer that funnels into MCP III. In the latter case one might expect to find analogous first-stage transducers for other receptors. The former possibility suggests that MCP I and II should interact directly with the receptors they serve.

It seems evident that much effort will now go into the purification and characterisation of MCPs. The proteins must interact with receptors or first-stage transducers on one hand and with the enzymes of methylation and demethylation and perhaps additional *che* products on the other. An understanding of the mechanisms of signal transduction and adaptation will require a detailed elucidation of those interactions. □

bound-states are seen. Above 3.8 GeV there is a significant increase in the rate of hadron production. Then, as more and more spare energy becomes available, the two quarks have to get faster and jets are formed. Just below 10 GeV the pattern seems to be repeating the narrow 'upsilon' and 'upsilon'' 'bottomonium' states being found (see *News & Views* **273**, 705; 1978). At the new PETRA energies there is just a suggestion of extra hadron production due to  $b$  anti- $b$  production and a slight check in the rate of increase of two-jet properties, though the underlying trend is dominated by the lighter quarks with  $2/3$  charge. The real interest over the next few months will come as energies are increased from 17 to around 30 GeV. Many theorists expect a heavy  $2/3$  charge quark called  $t$  ('top') which should give another clear step in the rate of hadron production along with a sudden dilution of the two-jet structure. When such changes are seen it will be possible to search back in energy to the  $t$  anti- $t$  threshold and then to look for  $\psi$  or 'upsilon'-like 'toponium' states just below it.

One of the collaborations at PETRA, using the PLUTO detector, has reported another important result which especially interested physicists who visited DESY in the first week of April to continue the studies for a very high energy European electron-positron collider (LEP) (see *News & Views* **275**, 482; 1978). The PLUTO group has seen 100 events caused by the collision of pairs of virtual photons, one contributed by the electromagnetic field of an electron in the electron-beam, the other from the field of a positron in the opposed beam. Such events are predicted to be much more frequent at higher beam energies and they may form an unpleasant background to the important single photon annihilation processes. But they also open a new window onto processes which have not yet been studied so their observation at PETRA will encourage those who have been planning special experiments to look at these reactions.

The most important recent result comes, however, not from PETRA but from DORIS. After last year's announcement of their observation of the 'upsilon', the PLUTO group moved the detector to PETRA but continued to analyse data from DORIS. These show that hadron production by way of the 'upsilon' seems to be dominated by three jets rather than two. This has been demonstrated by statistical analyses of particle distribution (see *DESY preprint* 78/71) and they have now reported an 'energy-flow' diagram which shows clear signs of three-jet structure which they claim cannot be

## News from PETRA and DORIS

from David J. Miller

THE new electron-positron storage-ring PETRA has now been working for about 6 months at the DESY laboratory near Hamburg and the first physics results have just been reported. They are interesting but not unexpected. Three detectors were used, each manned by a different international collaboration of universities and national laboratories. All see the same sorts of effects, but with different sensitivities (see for example *DESY preprint* 79/11 and papers in recent issues of *Phys. Lett.*). Data have been collected at total energies of 13 GeV and 17 GeV. There is evidence for the increase, as energy is increased, of the tendency for strongly-interacting particles (hadrons) to be produced in a pair of 'jets'. A jet is a cluster of particles all moving in roughly the same direction and all generated, according to present theories such as quantum-chromodynamics (QCD) (see *News & Views* **277**, 349; 1979) by one fundamental parent—a quark or a gluon. In QCD the underlying interaction is supposed to be between quarks and gluons; the quarks behaving like particles when within a femtometre of each other but needing to convert

themselves into pions and other genuinely free hadrons when they try to separate. Fast gluons are expected to form jets in a similar way. As well as their clear two-jet structure, the PETRA data on the interaction rate and on the number of particles produced conform to the pattern which has been observed at lower energies with the SPEAR storage-ring in California and up to 10 GeV with the DORIS rings at DESY.

All hadron production by electron-positron annihilation can still be explained by production of pairs of quarks and antiquarks from the set  $u$ ,  $d$  (the quarks in normal matter such as protons or neutrons),  $s$  (the strange quark),  $c$  (the charmed quark) and  $b$  (the heavy 'bottom' quark). Since the production process goes by way of a single intermediate photon the contribution due to each type of quark depends on the square of its electromagnetic coupling—that is, the square of its charge. The bottom quark appears to have  $1/3$  times the electron charge so it is produced at only one quarter of the rate at which the  $2/3$ -charge  $c$  quark is produced. The threshold energy for  $c$  anti- $c$  production is about 3.8 GeV. Below that the narrow  $\psi$ -J and  $\psi'$  'charmonium'

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reproduced by any other simple mechanism. Some theorists are hailing this result as the proof that gluons exist, though many people are not fully convinced.

If the  $\psi$  is produced by way of a single photon then it must have negative charge-conjugation parity (C-parity). Since the  $\psi$  is too light to decay to the  $b\bar{b}$  continuum it can only decay to quarks if the weak interaction turns a  $b$  into a lighter  $c$  quark, but that is a very slow process. In the meantime there is predicted to be a high probability that it can decay into gluons. But gluons are like photons in a number of ways. In particular, they also should have negative C-parity, so the  $\psi$  can only conserve C-parity if it decays to an odd number of gluons. Energy-momentum conservation forbids decay to one gluon and the phase-space probability is small for five gluons or more. Sceptics claim, correctly, that other three-jet models can be constructed, but QCD does predict that three gluon jets should be seen and the PLUTO results must therefore be taken seriously as at least circumstantial evidence for the existence of gluons inside hadrons.  $\square$

## Ten years after . . . a decade of lunar science

from C. T. Pillinger

THE 10th anniversary meeting of the Lunar and Planetary Science Conference\* met under the shadow of recent budgetary constraints imposed by the US Congress. The growing 'planetary' nature of the conference was marked; indeed discussion on new data from lunar soils has diminished substantially from its originally dominant position. Data from new planetary missions, meteorites and studies of fundamental processes on the Moon attract more interest from today's planetologist.

A highlight of the conference was the presentation of new results from the Viking, Voyager and Pioneer missions. A short 3-D film of Mars, created from elegant computer techniques by E. Levinthal (Stanford University), preceded spectacular close-up pictures of Jupiter and the Gallilean satellites, providing exciting new venues for geological speculation. The volcanic activity on Io, and apparent tectonic effects on Ganymede, will no doubt provide the basis for much discussion at future meetings. The composition of the atmosphere of Venus, with its apparently 'solar' rare gas

abundance (see *News & Views* 278, 777; 1979), leads one to speculate as to how radically our current ideas of planetary formation may have to be revised over the next 10 years.

As pointed out by S. R. Taylor (Australian National University, Canberra), 10 years study of the Apollo samples has failed to achieve consensus on the origin of the Moon, although considerable constraints can be inferred. Existing disagreements continued over the criteria for estimating siderophile element abundances indigenous to the highland crust and the whole Moon. Delano and Ringwood (Canberra) disputed the elemental correlations calculated by the group of E. Anders (University of Chicago). The importance of the argument is the validity of the assertion by Ringwood that the Moon was derived from the Earth's mantle after formation of the Earth's core, as opposed to condensation as a discrete body. A third position was adopted by Wanke *et al.* (University of Mainz) who, on the evidence of Co/(Fe + Mg) ratios, favoured a genetic relationship between the Earth and Moon.

Continued studies of lunar drill core samples have clarified ideas concerning reworking and deposition processes in the lunar surface layer, known in lunar parlance as 'the regolith'. Soil exposure indices have been based on a number of irradiation effects and petrographic particle size data. However, the relative importance of erosion compared with deposition processes (for example sputtering, impact volatilisation and deposition, and micrometeorite erosion) continues to be the subject of lively debate.

Wieler *et al.* (University of Zurich) showed that 0.5 billion year old soils had similar solar rare gas ratios to the present; no information on the solar wind flux in the past was forthcoming, although various workers have postulated an increased flux of varying isotopic composition in earlier irradiation. The magnitudes of the postulated changes seem to be at variance with existing models of solar processes.

Meteoritic studies have benefited from techniques developed for the lunar programme, and the growth of such investigations was evident. The Allende meteorite in particular has been subjected to intensive study. The Caltech group presented a detailed study of a new hibonite-rich inclusion (HAL); hibonite is considered to be one of the highest temperature major element condensates from the solar nebula. This inclusion consisted of an unusually pure hibonite core and several rims of lower-temperature

phases. Comparison with other Allende Ca, Al-rich inclusions implied a large  $^{26}\text{Mg}$  anomaly; however, this was not found, suggesting a low  $^{26}\text{Al}$  content for HAL. Ca isotope shifts in all phases were found to be large but uniform, and small nonlinear effects attributed to a nuclear origin. HAL thus qualifies as a new Allende isotopically-anomalous inclusion. Jessberger *et al.* (University of Heidelberg) reported a very high  $^{39}\text{Ar}/^{40}\text{Ar}$  apparent age of over 5 billion years, which can either be interpreted as a very old component, or a possible  $^{40}\text{K}$  isotopic enhancement. The latter possibility should be resolved by work now under way in Mainz. The existence of very old inclusions ( $> 5$  billion years) would imply that either these inclusions originated from outside the condensing solar nebula, or that the currently accepted age of the Solar System of 4.5 billion years needs to be re-assessed.

Strongly nonlinear kinetic isotope fractionations of the three stable isotopes of oxygen have been produced in the laboratory. G. Arrhenius (La Jolla) suggested this effect as another source of the nonlinear isotopic behaviour, to complement the nucleosynthetic processes. The latter were proposed to explain the original discovery of such anomalies in oxygen by R. N. Clayton in 1973.

Recent and previous discoveries of isotopic anomalies place constraints on possible models of the origin of the Solar System. However, considerable latitude is still available, as a number of apparently contradictory models were discussed at length mainly by D. D. Clayton (Rice University) and A. Cameron (Harvard University).

An interesting new sample collection method, which may be important to future research, is the 'cosmic muckrake' of Brownlee and others. Using a magnetic rake dragged across the ocean floor, large quantities of extraterrestrial material can be recovered. In a typical day's operation, the device processed  $\sim 10^7$  kg of sediment, and separated about half a million particles, many of which were identified as extraterrestrial. Debris from nearly every type of object which has collided with Earth over the past  $10^9$  years might be collected.

The few contributions mentioned outline some promising research emerging in planetary science. The emphasis on the Moon a decade ago has given new impetus to the older science of meteoritics; additionally, new information from hitherto inaccessible objects is now becoming available.  $\square$

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\*Held at the Johnson Space Center, Houston on 19-23 March.

# review article

## Games parasites play: how parasites evade immune surveillance

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*Parasites have evolved an extraordinary variety of mechanisms for surviving in the face of the natural and acquired immune responses of their hosts. A selection of the mechanisms serves to illustrate the challenge involved in developing strategies for immunising against parasites.*

THE games parasites play are, in fact, a serious matter (see refs 1–11 for reviews). It is estimated that between 500 million and 1,000 million people, primarily in the developing countries, suffer from tropical diseases and parasitic infections. Table 1 indicates current estimates on the prevalence of some parasitic diseases. In addition to the diseases of man, millions of cattle die of parasitic diseases each year (Table 2) and at least 700 million are estimated to be at risk. In Africa alone, 7 million square kilometres of grazable land capable of supporting 120 million head of cattle remain largely unproductive chiefly because of two parasitic diseases—trypanosomiasis and East Coast fever.

There is currently a resurgence of interest in tropical diseases, and in the possibility of providing immunological protection against many of them. The challenge to the immunologist, in trying to engender resistance or immunity to these parasites, is to improve the natural capability of the host's immune system, given that the survival of parasites and the extent of their ability to live in the host species reflect the fact that they have evolved mechanisms for surviving in the face of the best the body has to offer in the way of natural and acquired immunity.

In the simplest of terms, the general ground rules of the game may be sketched as follows: If parasites totally eluded the immune response and were sufficiently virulent, they would kill their hosts on whom their survival depends and preclude their own survival. Conversely, if they were too easily destroyed by the immune response, their survival would be similarly jeopardised. Consequently, immunity and escape from surveillance are relative phenomena which exist in balance and tension.

My intention here is to outline some of the mechanisms currently put forward to explain escape from surveillance. From this rapidly accumulating knowledge there should arise some new approaches to the prevention of parasitic diseases. It is to be expected that as these approaches are applied, they will be recognised by the parasites as selective pressures to be overcome, and they will devise even more sophisticated mechanisms for eluding the host's immune response. But one may hope that the time frame of evolution will be a great deal longer than the time frame required to bring most infectious diseases under some control.

### Old-time genetic engineering

In West Africa, there is considerable variability in susceptibility among various breeds of cattle to infections produced by *Trypanosoma congolense*. Cattle introduced to Africa from Europe during the time of the Roman Empire as well as native zebu cattle are highly susceptible to this parasite. Indigenous West

African short-horned cattle such as the n'dama and muturu seem to be naturally much more resistant<sup>12</sup>. This is an example of what is generally termed 'natural immunity'. The spectrum of natural immunity is illustrated by the fact that there can be some parasites such as *Trypanosoma b. brucei* and *Trypanosoma cruzi* within a single classification which have enormous host ranges and are capable of infecting and growing in virtually all domestic mammals; others, such as *T. simiae*, can produce fulminating infection in only a few, apparently unrelated, species such as monkeys and pigs; and there may be others which produce infections with more limited host range, such as the exclusive infection of cattle by *Theileria parva* or certain species of monkeys by the monkey malaria agent, *Plasmodium knowlesi*. The mechanisms underlying these host restrictions remain largely unknown; yet were such mechanisms understood, it might be possible to learn much about the parameters which govern host-parasite interactions.

In spite of the current enthusiasm about immune response (Ir) genes, in a wide variety of experimental bacterial, viral and parasitic infections studies in inbred mouse strains, it has not yet been established that natural host resistance is primarily dependent on genes associated with the major histocompatibility complex. Within a host species, however, there may be striking genetic differences in susceptibility to parasitic infection. This is perhaps best illustrated by studies on two protozoa that cause quite different diseases in man—*Leishmania donovani*, the causative agent of visceral leishmaniasis or kala-azar and *Trypanosoma cruzi*, which produces Chagas' disease in Latin America. When various inbred mouse strains were studied for susceptibility to *L. donovani* by Bradley<sup>13</sup>, C3H/He mice seemed to be most resistant and C57B1/6, most susceptible. By appropriate crosses it was possible to show that there was a single major autosomal dominant gene which controlled resistance, and that this gene was not H-2-linked. Studies in our

Table 1 Some parasitic diseases in man

	Estimated prevalence
Hookworm	700,000,000
Trachoma	400,000,000
Schistosomiasis	200,000,000
Malaria	150,000,000
Filariasis	200,000,000
Chagas' disease	12,000,000
Sleeping sickness	Unknown
Leishmaniasis	Unknown

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laboratory on resistance to *T. cruzi* in the same strains of mice, revealed an almost reciprocal pattern of susceptibility<sup>14</sup>. Here the C3H is susceptible and the C57B1, relatively resistant. Again we have found resistance to be unlinked to H-2. It is obviously of great interest to pursue the mechanisms by which these major autosomal genes control resistance. In Bradley's studies, there was some evidence that an H-2-linked gene exerted a secondary influence on resistance following infection. In this regard studies of patterns of the major human histocompatibility complex (HLA) antigens in Sardinia by Piazza *et al.*<sup>15</sup> showed significant differences in the HLA-B locus antigens between inhabitants of the highlands, an area in which malaria was essentially non-existent, and of the lowlands, an area in which malaria flourished until recently. This suggests that there may have been a selection for certain HLA-B-linked, possible Ir gene functions by the malarial parasite.

The converse situation, in which the outcome of infection is modified by the genetics of the parasite, is illustrated by three extraordinarily different disease entities of man in three different parts of the world caused by *Leishmania*, a genus of protozoan parasites which grows exclusively in macrophages in man and certain animal reservoir hosts. *Leishmania donovani* causes the systemic, progressive non-healing disease, visceral leishmaniasis, known in South Asia as kala-azar; *Leishmania tropica* causes Oriental sore found mostly in the Middle East and which is a local, cutaneous, self-healing lesion; and *Leishmania brasiliensis*, which causes mucocutaneous leishmaniasis in Central and South America with its most fulminating form known as espundia, a disease in which the parasite destroys the soft tissue and literally makes its victims faceless. Our lack of knowledge about the genetic control of pathogenesis makes it impossible to understand how three such different diseases can be produced by related organisms that grow in the same cell type. Nor why the same organisms inhabiting different host species produce different patterns of disease than in man.

### How parasites change their spots: antigenic variation

There is no more striking example of successful adaptation of a parasite to the host's immune response than that exhibited by African trypanosomes, which live entirely extracellularly, particularly in the blood stream and lymph of ungulates or man where they cause sleeping sickness. As long ago as 1907 Massaglia (see ref. 16) observed that following infection through the bite of the tsetse fly, the numbers of parasites in the blood fluctuated periodically. He suggested that this succession of parasitaemia, remission and recrudescence was due to the destruction of trypanosomes by host antibody and the emergence of parasites of different antigenic constitution. The course of these waves of parasitaemia in a single patient described in 1910 is illustrated in Fig. 1. Human sleeping sickness is caused primarily by two subspecies of *Trypanosoma brucei*, namely *gambiense* and *rhodesiense*. Each wave of parasitaemia is followed by the production of antibodies, generally agglutinins or lysins, which are specific for an individual variant, that is the antigen type of the previous wave of parasitaemia. Antigenic variation has also been described in the genus *Plasmodium*, which causes malaria, and in *Babesia*, tick-borne parasites causing a malaria-like disease.

What are the possible genetic and regulatory mechanisms which could explain antigenic variation? The simplest hypothesis would be that most infections contain a mixed population of parasites comprising several strains, and as the predominant

strain grows and is killed, there is simply outgrowth of another variant type present in the original inoculum. This hypothesis was essentially disproved by experiments in which single trypanosomes were cloned and maintained in immunologically compromised animals and antigenic variation was not seen. When transferred to conventional animals, even though the entire population was derived clonally from a single organism, antigenic variation emerged<sup>17</sup>.

A second hypothesis invokes mutation, possibly involving expression of small determinants in the molecule which are immunodominant and which could vary randomly to elude the immune response. A mutation frequency of  $1.2 \times 10^{-5}$  would explain the appearance of variants in a clone at 3–4 day intervals. But Gray showed that antigens of a clone tend to appear in an apparently programmed sequence<sup>18</sup>. Thus, display of variant antigens A-B-C-D-? appears to be non-random, and passage of variants at any stage back through the tsetse fly results in reversion to the basic antigenic variant types of the strain. It is difficult to explain a programmed sequence on the basis of simple random mutation.

The mechanism believed to be most relevant to African trypanosomes is adaptive phenotypic variation, in which the parasite has a finite number of genes which can code for its surface antigens and which it can switch on and off in regular sequence. As gene activation and translocation is one of the central problems in molecular biology, the African trypanosomes provide an extraordinarily interesting model for study.

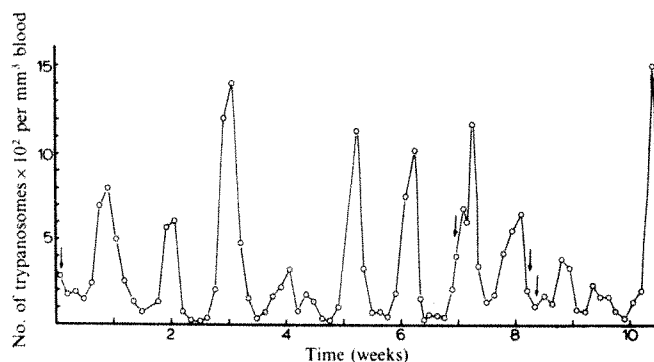


Fig. 1 Parasitaemia occurring in a patient with African trypanosomiasis. From Vickerman<sup>16</sup>.

What is the nature of the antigens that vary? Cross<sup>19,20</sup> has demonstrated that the outer surface of *Trypanosoma b. brucei* contains a coat or glycocalyx that is composed principally, if not exclusively, of the variant-specific glycoprotein antigen. This antigen has a MW of 67,000, and constitutes 10% of the entire cell protein and 30% of the soluble proteins. The variant glycoprotein molecules contain between 7 and 17% carbohydrate attached to the portion of the molecule associated with the plasma membrane. About  $7 \times 10^6$  molecules are found per trypanosome, a number probably sufficient to cover the entire surface of the parasite, and it is possible that this coat, which projects 12–15 nm from the plasma membrane, may serve as a spacer or barrier sufficient to prevent antibody to common membrane antigens from ever reaching the plasma membrane and lysing the organism. Amino acid sequencing of the N-terminal ends of four isolated purified variant glycoproteins revealed essentially no structural homologies. Each had a unique sequence, and there was no obvious evidence for deletions or frameshifts, thereby apparently eliminating the simple mutational hypothesis for antigenic variation.

Does antibody select or induce the antigenic variants? As early as 1909 Ehrlich found that trypanosomes incubated with homologous antiserum and then injected into mice produced a parasitaemia of a different variant type. As mentioned before, passage of clones of trypanosomes or variants in immunologic-

Table 2 Some parasitic disease of cattle

	Estimated annual mortality
African trypanosomiasis	3,000,000
Anaplasmosis	1,000,000
East Coast fever	500,000
Babesiosis	250,000

ally incompetent animals results in clonal fidelity. Gray<sup>21</sup> was able to perturb the programmed antigenic succession in the rabbit by passively protecting animals against four variants of a strain and then challenging with that strain. The rabbits then produced antibody to a fifth variant when challenged with the original strain.

It has become possible for the first time to cultivate the bloodstream form of the African trypanosomes *in vitro*<sup>22</sup> using a bovine cell feeder layer, and it is now possible to analyse *in vitro* molecular mechanisms of genetic and regulatory control underlying antigenic variation. Doyle *et al.*<sup>23</sup> followed 19 clones of a variable antigen type of *T. b. brucei* *in vitro*, and, in the absence of antibody, observed new variants arising spontaneously in nine clones, with a frequency of approximately  $10^{-3}$ – $10^{-4}$ . In the absence of immunoselection *in vitro*, the ability to detect variant types was related to their ability to outgrow the initial population, suggesting that two mechanisms of selection for variants are likely to be operative *in vivo*, altered growth rates and immunoselection. It remains to be established whether antigenic variants from a clone occur randomly or not, and whether antibodies affect the variation frequency or merely select for spontaneously occurring variants. Nevertheless, the high frequency of spontaneous variants observed in these *in vitro* studies can be considered to support mutational hypotheses for antigenic variation, as the accumulation of large numbers of mutations in the genes coding for surface antigen could lead to production of variants of markedly differing surface antigenicity and amino acid sequences.

As another possible mechanism of antigen modulation it is important to consider the possibility that parasites can 'cap' and shed their surface antigens to elude the immune response. Doyle *et al.*<sup>24</sup> have shown that within minutes after exposure of leishmania to fluorescent antibodies at 37 °C, the parasites shed their surface antigens and become refractory to the effects of immune sera and complement. It is not known whether capping occurs *in vivo* and represents a significant mechanism for escape of protozoa from immune attack.

### Antigenic mimicry and concomitant immunity

While the term 'concomitant immunity' is most familiar in the context of a tumour-bearing animal in which an autochthonous transplant of the primary tumour is rejected while the primary tumour grows progressively to kill the animal, there is a precisely analogous phenomenon which occurs during infection with a number of parasites. Concomitant immunity<sup>25</sup> is a form of acquired immunity in which the established parasitic infection persists long after resistance has developed against a challenge infection. In contrast, 'premunition' is used to describe the situation in which infection is suppressed although not eliminated by the host<sup>26</sup>.

Concomitant immunity is well documented in schistosomiasis, a chronic disease caused by the trematode genus *Schistosoma*. *S. mansoni* and *S. japonicum* reside in the portal and mesenteric systems and produce a hepatosplenic disease. *S. haematobium* causes a disease of the urinary tract. Adult schistosome worms can reside in the mesenteric venules for many years with little evidence to indicate that they stimulate any immune response at all. However, if adult schistosome worms are transferred to portal veins of monkeys which have never experienced the immature migrating forms, the monkeys become capable of resisting infection when challenged with the immature larval form (cercaria) even though they could not reject the transferred adults<sup>27</sup>.

How can this type of concomitant immunity be explained? One possibility is that the parasite, by natural selection, has evolved some antigenic determinants similar or identical to those of the host. One difficulty with this hypothesis, at least with respect to schistosomes, is that the organism can live in a variety of mammalian hosts, and it is unlikely that an organism would have evolved major antigens common to all hosts. An alternative hypothesis, originally suggested by Smithers and Terry<sup>25</sup> is that schistosomes adapt to the range of hosts in which they live

by appropriating some host molecules onto their surface layer or tegument. This has been amply demonstrated for *S. mansoni*<sup>27</sup>. Worms grown in both mice and gerbils were transferred either to normal rhesus monkeys or to monkeys immunised against the erythrocytes of mice or gerbils. The adult worms transferred to normal monkeys resumed their egg laying and were viable after about 5–6 weeks. Adult worms transferred from mice to monkeys immunised against mouse erythrocytes failed to establish themselves and were killed. But monkeys immunised against mouse erythrocytes failed to inhibit the growth of adult worms grown in gerbils. These experiments suggested that the worms grown in mice had acquired mouse antigens on their surface.

In order to explain concomitant immunity, it is necessary to argue that the host antigens are acquired by the adult worms but are not found on the immature forms, as the immune response of the chronically infected host can kill the immature forms. Clegg *et al.*<sup>28</sup> demonstrated that the most immature form, the cercaria, is not killed by the monkeys immunised against mouse erythrocytes, and hence does not appear to have host antigens, but that by 7 d the intermediate stage of the parasite, the schistosomulum, has already acquired host antigens.

What are the host antigens acquired by these organisms? Schistosomula cultured with human type A, type B or type O erythrocytes have been shown to acquire A, B and H blood group antigens respectively on their outer layer<sup>29</sup>. Blood group Rh or MN determinants are not found on the schistosomula but several other host serum proteins have been found. Most exciting is the recent evidence of Sher *et al.*<sup>30</sup> that schistosomula can acquire mouse histocompatibility antigens, the major determinants of immunological recognition of 'self', and this offers an exquisite mechanism for mimicry of 'self'. Intriguing as these data are, there is as yet no conclusive evidence establishing that 'mimicry' is the mechanism primarily responsible for the failure of adult worms to be rejected by the host.

### Learning to live in macrophages

From the time of Metchnikoff, macrophages have been described as highly phagocytic scavenger cells capable of killing and degrading a wide variety of microbes. Nevertheless a number of microorganisms including *Mycobacterium tuberculosis*, *Mycobacterium leprae*, and protozoa of such genera as *Toxoplasma*, *Besnoitia*, *Leishmania* and *Trypanosoma cruzi* survive and grow inside macrophages for part, or all, of their stay in the mammalian host.

Extracellular particles which are phagocytised by macrophages enter by invagination of the plasma membrane and appear in phagocytic vesicles<sup>31</sup>. There follows a fusion between the phagocytic vesicle and primary lysosomes, known to contain degradative enzymes, and probably peroxisomes as well, to form secondary lysosomes. In addition, fusion between secondary lysosomes and phagocytic vesicles is also known to occur. At least three different mechanisms can be defined from studies *in vitro* to account for the ability of parasites to evade killing by the macrophage, although the molecular basis for these phenomena and their relevance to the survival of parasites *in vivo* remain unclear.

(1) Failure to fuse: A number of parasites have evolved mechanisms to prevent fusion of macrophage lysosomes with the phagocytic vesicle containing the invader. Human virulent tuberculosis grows in vesicles within macrophages, but these fail to fuse with lysosomes<sup>32</sup>. Fusion can be induced by opsonisation (coating viable mycobacteria with antibody) but the organism retains its viability<sup>33</sup>.

A very similar pattern has been recorded for *Toxoplasma gondii*<sup>34</sup>. Once again, viable organisms were readily ingested by macrophages *in vitro*, but there was a failure of lysosomes, for example secondary lysosomes labelled with Thorotrast, to fuse with the phagocytic vesicle. Opsonisation of the parasite with antibody permitted fusion of secondary lysosomes with the phagocytic vesicle. In this case, the effect of antibody is to facilitate killing of the *Toxoplasma*. The mechanisms by which



the living parasites are able to block lysosomal fusion in host cells remain largely unknown.

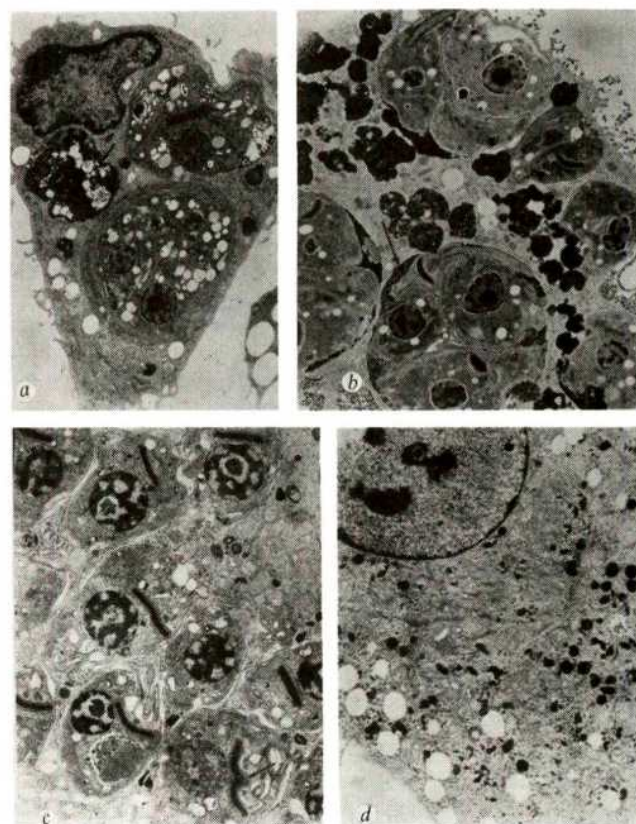
(2) Resistance to killing: As mentioned above, when *M. tuberculosis* is opsonised, lysosomal fusion occurs, but the mycobacteria are perfectly capable of surviving within the lysosome. *M. lepramurium* also grows in vesicles which fuse avidly with primary and secondary lysosomes even without opsonisation<sup>32</sup>. Similarly, leishmania grow exclusively in macrophages, and survive and multiply in lysosomes even after fusion has occurred<sup>35</sup>.

A subtle example of survival in macrophage lysosomes is provided by two species of *Leishmania*. *L. enrietti* produces a disease similar to human cutaneous leishmaniasis or Oriental sore in the guinea pig; *L. tropica* produces a similar disease in some strains of mice. When macrophages from mice sensitised to *L. enrietti* were activated and then challenged *in vitro*, Mauel and Behin<sup>36</sup> found them to be resistant to *L. enrietti*. However, when similar procedures were used for activating guinea pig macrophages, *L. enrietti* was not killed. In contrast, activated guinea pig macrophages were highly resistant to *L. tropica*, but failed to resist challenge with *L. enrietti*. Thus, these leishmanias have developed quite specific, but unknown, mechanisms for eluding the destructive capability of macrophages from the species which they normally infect, while being susceptible to the killing mechanisms of activated macrophages from animal species which are resistant to infection by them. An elegant control in these experiments was the simultaneous infection of activated guinea pig macrophages with *L. enrietti* and *Listeria monocytogenes*, to ascertain whether the leishmanias survived by nonspecifically blocking the macrophage cytotoxic mechanisms<sup>37</sup>. The results obtained were surprising in that, in the same phagosomes, listeria were killed but the leishmanias survived. Thus, there is an extraordinary selectivity in the evolution of resistance to macrophage killing by *Leishmania*.

(3) Escape from the lysosomes: In studies on the interaction of *Trypanosoma cruzi* with normal and activated murine macrophages<sup>38-40</sup> it was observed that within 2 h virtually all trypanosomes were in phagocytic vesicles, and that lysosomal fusion almost invariably occurred (Fig. 2b). Resident unstimulated peritoneal macrophages were as effective as BCG-activated macrophages in killing and degrading a high proportion of the infecting organisms within the first 24 h. However, when infected cultures were examined at 72-96 h, a remarkable picture was seen. Resident macrophages were found to have large numbers of trypanosomes growing within the cytoplasm, and when the Thorotrast labelling technique was used, virtually none of these parasites could be found in a vesicle containing the lysosomal marker (Fig. 2c). They appeared to be able to escape from their precarious state within the lysosome, and take up residence in the cytoplasm, where the macrophage has no specialised mechanism for killing foreign invaders. In contrast, by 3-4 d after infection, macrophages nonspecifically activated by BCG had essentially killed and degraded virtually all of the parasites; only debris was seen (Fig. 2d). Recently, Noguiera and Cohn<sup>41</sup> have been able to activate resident mouse macrophages *in vitro* to kill *T. cruzi* using products of activated lymphocytes.

### Jamming the immune response

One of the intuitively obvious adaptive mechanisms by which parasites could elude the normal host response would be by suppressing the immune response itself. Several organisms are known to use this method. For example, immunosuppression has been reported in hamsters infected with *Leishmania donovani*<sup>42</sup>, mice infected with *Toxoplasma gondii*<sup>43</sup> and rats infected with roundworm, *Nippostrongylus brasiliensis*<sup>44</sup>. However, by far the most impressive and clinically important instances of the immune suppression of the host response occur in African trypanosomiasis<sup>45-47</sup>, malaria<sup>48</sup> and kala-azar. In human sleeping sickness and malaria, elevated levels of IgM are characteristic, on occasion rising to 5 g per 100 ml<sup>49</sup>. In spite of extraordinarily high levels of IgM, 5% or less of these immuno-



**Fig. 2** a, Normal mouse peritoneal macrophages infected with epimastigotes of *T. cruzi* at a ratio of 1:1 after 2 h. Note that parasites are in the process of being digested (arrow).  $\times 2,500$ . b, BCG-activated macrophage infected with *T. cruzi* at a ratio of 10:1 after 2 h. Thorium dioxide is seen in secondary lysosomes and fusion with parasite-containing vacuole (arrow).  $\times 2,500$ . c, Normal mouse peritoneal macrophage at a 1:1 infection ratio after 96 h. Trypanosomes are present in cytoplasm where division has begun to occur (arrow). There is no evidence of lysosomal fusion.  $\times 4,000$ . d, BCG-activated mouse peritoneal macrophage after 192 h of infection with *T. cruzi*. There is no evidence of trypanosomes and some evidence of secondary lysosomes.  $\times 2,000$ .

globulins have been found to react specifically with antigens of *Plasmodium falciparum* or *Trypanosoma gambiense*, the parasites responsible for malaria and sleeping sickness in West Africa<sup>49</sup>. In both diseases the responses of patients to salmonella antigens and tetanus toxoid, and animals to sheep erythrocytes (SRBC), are markedly reduced<sup>46</sup>. In this paradoxical situation there is a heightened production of antibodies to the parasites with depressed humoral immunity to other antigens.

Several animal models have been developed to probe the mechanisms of this type of immunosuppression. For example, mice infected acutely or chronically with *Plasmodium berghei* malaria show a marked depression in development of antibody producing cells to SRBC in the Jerne plaque assay, the effect being more pronounced on the IgG plaques<sup>48</sup>. Even more striking suppression is seen in mice infected with *Trypanosoma b. brucei*<sup>47</sup>. Perhaps even more impressive than the suppression of antibody production is the marked increase in the background of antibody plaque forming cells to SRBC, generally at least a log higher in infected than in uninfected animals. Thus, infection with plasmodia or trypanosomes causes a marked increase in the background antibody forming cells not only to SRBC but also to donkey, horse or TNP-coupled SRBC. The high background and lack of specificity are strongly reminiscent of the observations made on mouse spleens treated 1-2 d before immunisation with a polyclonal mitogen, such as endotoxin



(lipopolysaccharide, LPS)<sup>50</sup>. There is an increase in immunoglobulin, an increase in polyclonal antibodies which account for the background, and a diminution in specific plaque-forming cells. These experiments suggest that one mechanism for inducing immunosuppression is a polyclonal mitogenic activity of the malaria and trypanosome parasites<sup>47</sup> causing many clones of B lymphocytes to undergo division and differentiation. Clearly other mechanisms, such as suppression by T cells, may be operative as well<sup>51</sup>.

Observations on the immunosuppression by parasites have implications beyond the survival of the specific parasite. It is well known that patients with sleeping sickness have an increased susceptibility to secondary bacterial infections, a problem recognised in some of the earliest studies on the disease. Children in the tropics with acute falciparum malaria also have high rates of bacterial infection; although it must be noted that many also have other parasitic infections, and frequently, protein calorie malnutrition and avitaminosis as well. Nevertheless, antibody responses in children to tetanus toxoid from villages where malaria prophylaxis is carried out are higher than those from comparable villages where malaria infection is frequent<sup>46</sup>. Furthermore it may not be coincidental that Burkitt's lymphoma<sup>52</sup> occurs mainly in geographical areas coincident with holoendemic malaria. It is not inconceivable that the malaria-induced immunosuppression could provide a favourable environment for development of lymphoma viruses. This possibility has received some experimental confirmation from the studies of Wedderburn in mice in which the incidence of lymphomas in mice infected with both malaria and murine leukaemia virus was dramatically higher than those receiving the virus alone<sup>53</sup>.

### Subversion of the immune system

While the above examples illustrate the ability of parasites to evade the immune system, there are at least two fascinating examples in which the immune system may be requisite for survival of the parasite. *Babesia* and *Theileria* species are protozoan blood parasites transmitted by ticks that can infect mammalian hosts. *Babesia* produces a malaria-like disease in cattle all over the world and *Theileria parva* causes a disease known as East Coast fever which affects cattle primarily in East Africa. *Babesia* Spp. occasionally produce disease in humans.

It has recently been found that *Babesia* seems to require complement activation in order to penetrate the erythrocyte in which it grows. C3 or C5 deficient mice or mice treated with cobra venom are protected from infection<sup>54</sup>.

Unquestionably, the most fascinating case of subversion of the immune system is that of *Theileria* species. These parasites enter lymphocytes and transform them into proliferating lymphoblastoid cells<sup>55,56</sup>. The intracellular parasite, in its large form called the macroschizont, divides by binary fission, and increases in number 10-fold every 3 d until almost all the lymphoid cells are parasitised<sup>57</sup>. During this process there is a marked lymphocytosis resembling the uncontrolled growth of a leukaemia or lymphoma. From about the tenth day of infection, increasing numbers of the macroschizonts break apart into a large number of microschizonts. In the process, host lymphocytes are destroyed and small infectious particles termed micromerozoites are released and invade red cells. There the parasite develops an intra-erythrocytic stage, after which there is a depletion of leukocytes and haematopoietic cells leading to death. In enzootic areas in East Africa, 40% of the calf crop may die from East Coast fever.

The most extraordinary aspect is that when lymphoblasts obtained from cattle infected with *Theileria* are put into tissue culture, they develop into continuously growing lymphoblastoid cell lines with clonal replication of both parasites and cells<sup>58</sup>. Sporozoites from the tick will transform normal bovine lymphocytes *in vitro*, thus *Theileria* species seem to be the only parasite other than oncogenic viruses capable of transforming lymphocytes into continuous growth.

Sceptics may argue that it is unlikely for a protozoan to induce neoplastic transformation of lymphoid cells, and that the parasite may be carrying an oncogenic virus. Yet it has recently been possible to cure the lymphoblastoid cells of the parasite using drugs<sup>59,60</sup> and they revert to small lymphocytes and stop growing. In any case, the continuous, persistently infected bovine lymphoblastoid cell lines, when transferred back to susceptible cattle, can engender protection to infection with *Theileria parva* or *Theileria annulata* in more than 80% of the instances<sup>61,62</sup>, and offer the possibility of a vaccine.

### Commentary

Several important new programmes in research in parasitic diseases have been initiated since 1976, prompted by increased awareness of the urgent health needs of developing countries and a commitment that basic biomedical research can provide new means for intervening in at least some of these diseases. Among these initiatives are the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, the Great Neglected Diseases Program of the Rockefeller Foundation and the establishment of the International Laboratory for Research in Animal Diseases in Nairobi.

Is there any reason to believe, in light of the subtle and sophisticated ways by which parasites elude immunological attack, that immunological approaches offer any promise? The tremendous advances in understanding basic cellular and humoral immune responses in model systems provide a basis for analysis of the mechanisms of natural and acquired resistance to parasites. The recent development of techniques to produce monoclonal antibodies by means of B-cell-myeloma hybrids (hybridomas) offers the possibility of identifying antigenic determinants, even minor ones, required for engendering resistance to infectious agents. Even if such protective antigens cannot be obtained in large amounts by cultivation of the parasites, as is the case for the blood form of the malaria parasite infectious for red cells or the common, non-varying metacyclic antigen found only on the insect form of the African trypanosomes, the monoclonal antibodies, together with recombinant DNA technology, could be useful for selecting clones of transformed *Escherichia coli* or yeast expressing the protective antigens of parasites which could be used in vaccines. Finally, the recent developments in soluble and liposome adjuvants offer increased possibilities for safe and effective enhancement of immune responses in man.

As in other areas in which basic scientific approaches are applied to disease-related problems, the study of immunity to parasites may provide a new and fundamental knowledge of far wider application than merely to the organisms or disease initially studied. In the early 1940s there was a major effort at the Rockefeller Institute to develop a vaccine against pneumococcal pneumonia. While Avery and his collaborators did not succeed in producing an effective vaccine, they did discover during these studies that DNA was the chemical basis of heredity<sup>63</sup>. Science too may have its parables.

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## articles

# Magnetostratigraphy, biostratigraphy and geochronology of Cretaceous–Tertiary boundary sediments, Red Deer Valley

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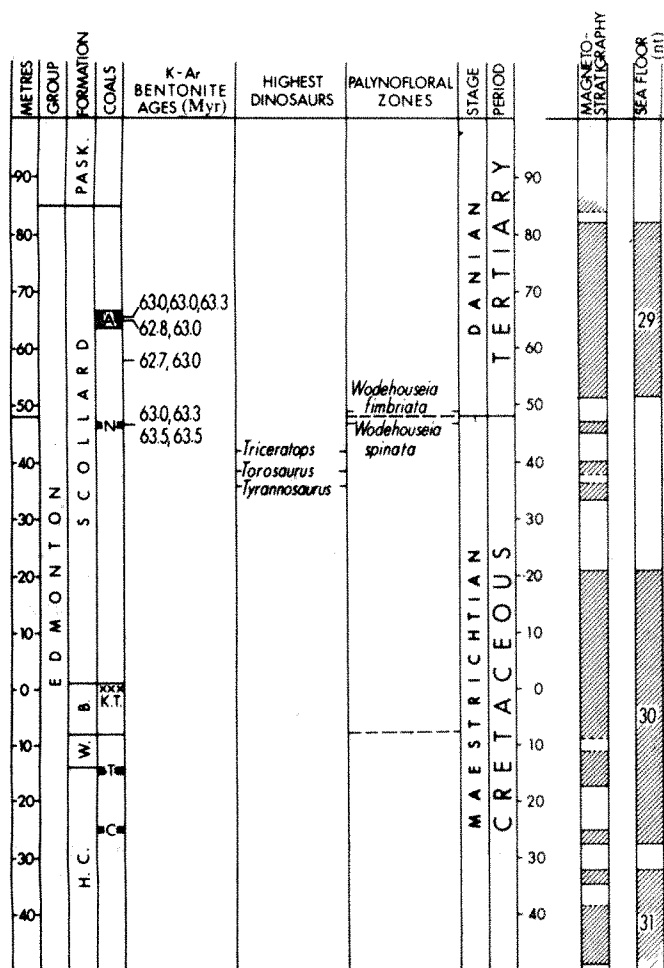
*Integrated magnetostratigraphic and biostratigraphic data for continental Cretaceous–Tertiary boundary sediments in Alberta allow a correlation with recognised sea floor magnetic anomalies 29 and 30. When compared with data from the marine Gubbio section in Italy, the correlation indicates that extinction of the dinosaurs on land was synchronous with foraminiferal extinctions in the sea which are equated with the Cretaceous–Tertiary boundary. We advocate that the base of anomaly 29 be tentatively accepted as the best worldwide physical approximation of the Cretaceous–Tertiary boundary. Eleven K–Ar dates on bentonite sanidines near this boundary yield a mean age of  $63.1 \pm 0.5(2\sigma)$  Myr.*

THE applicability of geomagnetic polarity reversals to worldwide stratigraphic correlation of sedimentary rocks has become accepted in the past few years. Interest in this application of 'magnetostratigraphy' is shown by the number of recent papers presenting slightly different polarity scales for the Mesozoic and Cainozoic<sup>1–3</sup>. Limitation of the accuracy of these scales is largely due to the scarcity of good radiometric dates.

The global demarcation of boundaries between major geological time units is very important. The Mesozoic–Cainozoic

boundary has caused a good deal of interest because of the evidence of worldwide extinctions and rapid evolutionary rates at this time. The organisms affected ranged from the largest to some of the smallest of the marine fauna (marine reptiles, ammonites, Foraminifera), and also included the largest continental animals, the dinosaurs, as well as some land flora. The search for causes of such widespread biotic changes has brought to light a large number of plausible explanations<sup>4</sup>. The choice between sudden catastrophic extraterrestrial events (supernovae), and somewhat slower terrestrial processes (global climatic changes), is hampered by the lack of sufficiently accurate and universally applicable chronological data on the contemporaneity of marine and continental extinctions. Correlation of the geomagnetic polarity reversals recorded in sedimentary rocks can provide the necessary accuracy on a worldwide scale. Two notable studies have been reported; one on a marine section near Gubbio, Italy<sup>5,6</sup>, the other on a continental section in the San Juan Basin of New Mexico, US<sup>7,8</sup>.

Accurate geochronology of the Cretaceous–Tertiary boundary is difficult because of a lack of explosive volcanicity in those parts of the world which were accumulating a record of fine-grained sedimentary deposits at this time. One of a few places where such volcanicity was prevalent, producing isotopically datable volcanic bentonites and tuffs, is the northwestern US and western Canada. This area was a swampy coastal plain flanking the western shore of a late Cretaceous mid-continental



**Fig. 1** Biostratigraphy, magnetostratigraphy and geochronology of the upper part of the Edmonton Group, Red Deer Valley, Alberta. B., Battle; W., Whitemud; H. C., Horseshoe Canyon; A., Ardley; N., Nevis; K.T., Kneehills Tuff; T, Thompson; C, Carbon. Cross-hatching denotes normal polarity intervals; dashed boundaries are more tentative than the solid line boundaries.

epieiric seaway with an active volcanic area further to the west. On this fluvial-deltaic lowland lived two of the greatest and the last of the dinosaurs, *Tyrannosaurus* and *Triceratops*.

We report here a study of geomagnetic polarity and K-Ar dating compared with dinosaur occurrences and recently published palynologic studies, carried out on an essentially conformable sequence of mudstones, sandstones, coals and volcanic ashes which encompasses the Cretaceous-Tertiary boundary. One aim of such a study is to obtain reliable dates from a section in which particular sea floor magnetic anomalies can be recognised, to provide data needed for improved temporal calibration of the older part of the marine sea floor anomaly sequence.

### Stratigraphy and palaeontology

The Red Deer Valley of south-central Alberta has long been known as a prime collecting area for late Cretaceous dinosaurs<sup>9</sup>; more recently it has been looked at by geochronologists<sup>10</sup> and palynologists<sup>11-13</sup>. The 100-m deep valley exposes within a distance of about 50 km the entire thickness (some 350 m) of the largely Maestrichtian Edmonton Group of nearly flat-lying fluvial-deltaic sediments (Fig. 1).

Detailed magnetostratigraphic analysis has been carried out on the upper 135 m of the Edmonton Group, concentrating particularly on the interval near the Cretaceous-Tertiary boundary as defined by a sudden major palynofloral change a few metres above the highest occurrence of dinosaur remains. The palynofloral change includes the abrupt disappearance of all species of *Aquilapollenites* (at least 10) except *A. spinulosus*, as

well as the disappearance of *Wodehouseia spinata*, *Cranwellia striata*, *Proteacidites* sp., and others. They are replaced by such species as *Wodehouseia fimbriata*, *Alnus trina* and *Carpinus subtriangula*<sup>13</sup>. An essentially identical palynofloral change takes place in Montana, Wyoming and the Dakotas at the Hell Creek (Lance)-Fort Union formational contact<sup>14,15</sup>. In this region the microfloral change also takes place a few metres above the highest occurrence of dinosaur bones, notably *Triceratops*.

In the Red Deer Valley of Alberta the palynomorph break occurs within a 2-m interval centred 1.5 m above a 20 cm bentonite near the top of the Nevis Coal seam. The stratigraphically highest known dinosaur remains still in place in the Red Deer Valley area are part of a scattered skeleton of a large carnivorous dinosaur in Sec. 10, Tp. 34, Rge. 22 West of the 4th Mer. This is the specimen first located by C.M. Sternberg in 1946 and recorded in his field notes as a scattered and broken skeleton of ?*Tyrannosaurus*. He collected part of a skull of a *Triceratops* recorded as occurring 20 feet higher and about 200 yards north of the tyrannosaurid. These specimens have been more recently classified as *cf. Tyrannosaurus rex* and *Triceratops albertensis*, respectively<sup>13</sup>.

The elevation of the *Tyrannosaurus* skeleton was apparently determined by Sternberg's party with an aneroid barometer, and the stratigraphic position determined by fitting this elevation into a more completely exposed section about 1 km to the south. Unfortunately, an error must have been made and the skeletons were inadvertently placed too high (stratigraphically) with respect to the Kneehills Tuff and the Ardley Coal seam. The portion of the tyrannosaurid which is still in place has been relocated and its stratigraphic position redetermined by one of us (J.L.). It lies 10.5 m below the top of the Nevis Coal seam. One assumes that the 20 feet stratigraphic separation between the two skeletons recorded by Sternberg is approximately correct inasmuch as the two sites are very close. The *Triceratops* is thus placed ~4.5 m below the Nevis Coal and 6 m below the Cretaceous-Tertiary palynomorph break. This *Triceratops* skull position is still the highest that we know to have been recorded in Canada.

In the Hell Creek Formation type area in east-central Montana at least three *Triceratops* skeletons have been recovered from an interval reported to be 9-17 m below the top of the Hell Creek Formation<sup>13</sup>. In the same area, two of us (J.L. and H.B.) found unreworked dinosaur bones within 4 m of the base of the Z coal which marks the palynofloral change and the Hell Creek-Fort Union contact. The position of the highest dinosaur bones in the type area of the Lance Formation in south-east Wyoming is shown to be about 3 m below the same palynomorph change at a lignite taken to mark the base of the Fort Union Formation<sup>15</sup>.

### K-Ar geochronology

The portion of the stratigraphic succession near the sudden palynofloral change contains pyroclastic bentonite beds suitable for uncontaminated sampling. Four bentonite horizons (+17.5, +17, +11 and -1 m from the palynofloral change) yielded transparent, unaltered sanidine of the requisite amount and purity for precise K-Ar dating. Duplicate bentonite samples were taken of three of the beds, and the sanidine separates were further split into two grain sizes to check on analytical reproducibility. Heavy liquid and magnetic separation procedures, followed by hand picking, enabled sanidine separates of at least 99.5% purity to be obtained.

Argon was extracted from the samples by flux-fusion *in vacuo*, mixed with pure <sup>38</sup>Ar isotope dilutant and purified by hot sponge titanium. Ar isotopic analysis was carried out on a Micro-Mass 6 gas mass spectrometer operating in the dynamic mode. Mass discrimination was determined by measurement of pure air argon in sequence with the Ar determinations in identical analytical conditions. Potassium was determined by isotope dilution using 99+ % enriched <sup>41</sup>K as the tracer. Decay

**Table 1** Analytical results from K-Ar dating of bentonite sandine

Sample, elevation (m), mesh size	$^{40}\text{K}^*$ (p.p.m.)	$^{40}\text{Ar rad}$ $^{40}\text{Ar total} \times 100$	$^{40}\text{Ar}/^{40}\text{K}$	Date* (Myr)
73-1(17.5) +150	10.97	97.6	0.003726	63.0
73-1(17.5) -150	9.68	96.3	0.003723	63.0
75-102(17.5) -80 +120	11.03	96.5	0.003745	63.3
75-100(17) -80 +120	10.82	94.8	0.003740	63.2
75-100(17) -120 +170	9.88	95.7	0.003710	62.8
75-104A(11) -80 +120	11.44	96.1	0.003725	63.0
75-104A(11) -120 +170	11.24	98.2	0.003704	62.7
(0) Palynofloral Cretaceous-Tertiary boundary				
74-1(-1) -80 +120	11.46	97.8	0.003726	63.0
74-1(-1) -120 +170	11.54	97.5	0.003744	63.3
75-400(-1) -80 +120	11.57	96.7	0.003753	63.5
75-400(-1) -120 +170	11.34	96.0	0.003753	63.5

\* Constants used:  $\lambda_B = 4.962 \times 10^{-10} \text{ yr}^{-1}$ ;  $\lambda_A = 0.581 \times 10^{-10} \text{ yr}^{-1}$ ;  $^{39}\text{K} = 93.2581 \text{ at.}\%$ ;  $^{40}\text{K} = 0.01167 \text{ at.}\%$ ;  $^{41}\text{K} = 6.7302 \text{ at.}\%$ .

constants for  $^{40}\text{K}$  and natural potassium abundances used in computations are those recommended by Steiger and Jager<sup>16</sup>.

The analytical results are given in Table 1. Using 68 Myr for the age of the base of the Edmonton Group<sup>17</sup>, the average rate of sedimentation for the Group is about 65 m per Myr; thus the upper bentonite horizons dated should differ in age from the lower horizon by about 0.25 Myr. This span is within the overall error of the K-Ar determinations, and the dates are averaged to give  $63.1 \pm 0.5(2\sigma)$  Myr. Taking into account  $2\sigma$  errors (precision) in the K-determination ( $\pm 0.8\%$ ), the Ar-determination ( $\pm 0.6\%$ ), the  $^{38}\text{Ar}$ -spike volumes ( $\pm 0.4\%$ ) and the decay constants ( $\pm 1.7\%$ ), the K-Ar date for the horizon of palynofloral change in the Red Deer section is  $63 \pm 2$  Myr. Considering indeterminant sources of error, such as sample quality and analytical manipulations, the  $\pm 2$  Myr should probably be at least  $\pm 3$  Myr.

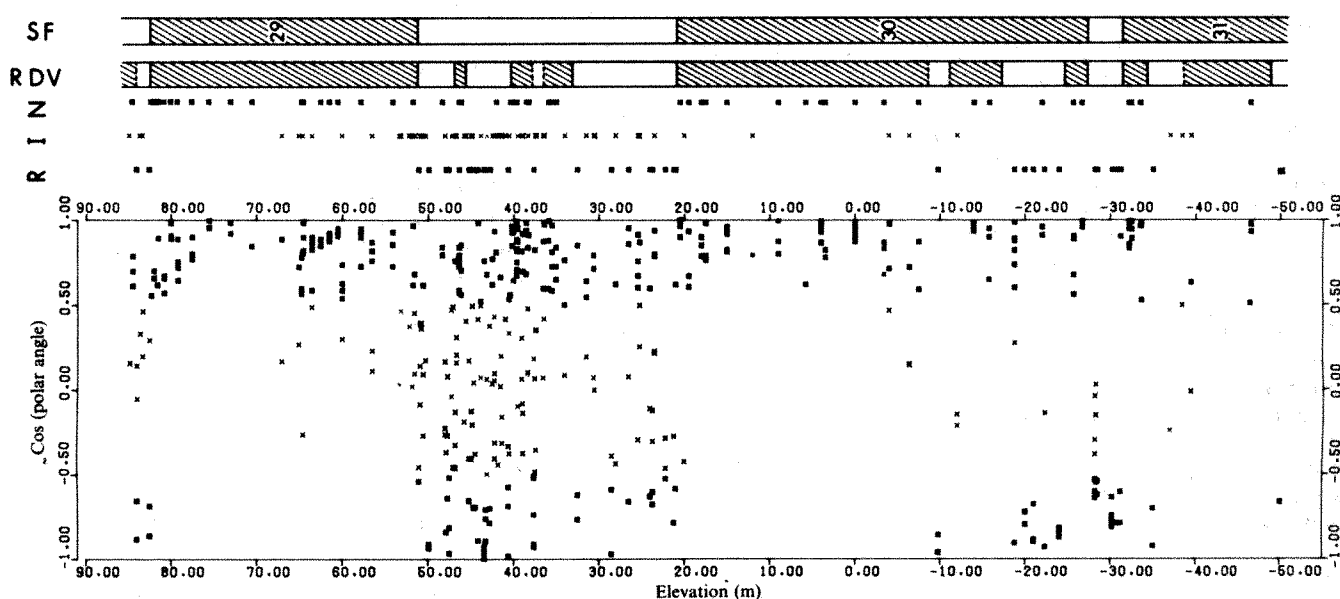
Careful optical assessment of sample purity showed essentially equal purity for fine- and coarse-grained splits of the sandine samples. Yet the K content (see Table 1) is invariably lower for the fine-grained fraction. The fine-grained fraction may be naturally lower in potassium, but the possibility of undetectable lower-K 'contamination' remains.

## Palaeomagnetic data

Approximately 600 samples were collected from some 170 stratigraphic levels (sites) covering 135 m of section. Normally three or four samples were taken at each site, occasionally more. Although the average vertical spacing is thus about 1 m, it varies from 3 m to 10 cm. Closer spacings are associated with polarity boundary levels, and were obtained after examination of preliminary samples. Samples are one-inch cylinders obtained by percussion coring.

The great majority of the remanence measurements were made with a Digico 'complete results' magnetometer, but a Schonstedt SM1 magnetometer was used for a few early measurements. Apart from the Kneehills Tuff and one other volcanic tuff bed, intensities of magnetisation were weak—generally below  $1.0 \times 10^{-6} \text{ e.m.u. cm}^{-3}$ . Routine alternating-field (a.f.) demagnetisation was carried out using the apparatus as described by McElhinny<sup>18</sup>. Completely standardised demagnetisation steps were not used as the material was variable, but peak fields of 75 and 100 Oe were most commonly used. Directional behaviour during progressive magnetic cleaning was highly variable, some specimens being relatively stable and others exhibiting large erratic angular changes. The least stable samples were eliminated by rejecting those associated with  $d\theta/dH > 0.5^\circ \text{ Oe}^{-1}$ , which reduces the data set to 455. The remaining remanence vectors represent 155 stratigraphic horizons and have been analysed as follows. The virtual geomagnetic pole (VGP) corresponding to each direction was calculated and compared to the well-established Upper Cretaceous-Lower Tertiary palaeomagnetic pole for North America (80–40 Myr,  $186^\circ \text{E}$ ,  $73^\circ \text{N}$ ,  $N = 11$ ,  $k = 51$ ,  $\alpha_{95} = 6^\circ$  (ref. 19). The angular differences between this pole and each VGP are here called 'polar angles' and are represented in Fig. 2 by the cosine of the polar angle.

Even after rejecting the most unstable material, considerable scatter remains. However, this scatter is not simply random noise—it is evident from Fig. 2 that a stratigraphic zonation exists. We argue that the profile represents a sequence of normal and reversed magnetozones but that secondary magnetic components acquired during the Brunhes epoch mask, to varying degree, the primary remanence of the reversely magnetised strata. An identical situation was recently described by Hillhouse *et al.*<sup>20</sup>. We adopt a modified version of their procedure



**Fig. 2** Magnetostratigraphy of the upper part of the Edmonton Group, and proposed correlation with sea floor magnetic anomalies (Kneehills Tuff is stratigraphic datum as in Fig. 1). See text for geomagnetic polarity assignment procedure. R, reversed; I, indeterminate; N, normal; RDV, Red Deer Valley; SF, sea floor.



for assigning polarity, namely: if all the VGPs at a given site have polar angles less than  $60^\circ$  the site is regarded as normal. If at least one VGP at a site has a polar angle greater than  $120^\circ$  the site is reversed. All other sites are regarded as indeterminate. Hillhouse *et al.* point out that these rules deliberately seek out reversed horizons and this is consistent with the hypothesis that these zones tend to be eliminated by recent magnetic overprinting. Indeed, it is still possible that certain indicated normal sites consist of entirely overprinted material, whereas the same is very unlikely to be true for reversed assignments. The choice of  $60^\circ$  as a cut-off value is arbitrary—we use it because it divides each palaeo-hemisphere in half. This procedure leads to the polarity profile shown in Fig. 2.

## Discussion

In Fig. 2 we have attempted to match the Red Deer Valley magnetozones with numbered sea floor anomalies. Recently published polarity scales place the Cretaceous–Tertiary boundary near the base of anomaly 29 (refs 1–3). The fairly long normal polarity zone which embraces the Ardley Coal seam (Fig. 1) thus corresponds to anomaly 29. The other relatively long normal zone (which includes the Kneehills Tuff) is then equivalent to at least part of anomaly 30, and our data suggest that the reversed magnetozones between 29 and 30 contains several short normal intervals.

It is more difficult to identify the short reversed interval which seems to be accepted as a diagnostic marker between anomalies 30 and 31. We have strong indications of at least two comparatively short reversals, either of which would seem to qualify. The lowermost reversal leads to the best relative time span for anomaly 30 versus anomaly 29 according to sea floor data<sup>1–3</sup>. Our measurements do not go low enough to enable us to use data from strata equivalent to anomaly 31. In the Gubbio marine section, the sampling interval of Roggenthen and Napoleone<sup>21</sup> allows several short reversals. The scale of the Lowrie and Alvarez<sup>6</sup> diagrams for the same section makes it difficult to assess their sampling interval, but the reversed zone they have located seems rather high in the section and may correspond to one of our two higher ones.

The presented pattern may still be somewhat oversimplified because we have not placed much weight on several possible short polarity intervals which are represented by more than one site in the unscreened data and are likely to be real, but may represent local geomagnetic 'excursions'.

One or more short normal polarity intervals below anomaly 29 seem to have shown up in other recently published results. Keating *et al.*<sup>22</sup> show a short normal in what may be this reversed zone at DSDP site 208. The data of Butler *et al.*<sup>7</sup> from New Mexico could also be differently interpreted by re-assigning the normal magnetozones they have called 28 to anomaly 29. This would allow correlation of the short normal they have called 29 with our short normal(s) in the reversed zone below 29 (Fig. 3). This re-assignment also removes the palaeontological discrepancies between the New Mexico, Alberta, and Gubbio, Italy sections with respect to the Cretaceous–Tertiary boundary and anomaly 29. Also, because the Cretaceous–Tertiary boundary in the Red Deer Valley is just below the base of anomaly 29 (Fig. 1), this assignment would fit Powell's<sup>23</sup> placement of the boundary in the San Juan Basin in his Kimbeto Member of the Ojo Alamo Sandstone = 'upper conglomerate' of Butler *et al.*<sup>7</sup>. Alternatively, the boundary may well be as low as the unconformity at the base of the conglomeratic member, as suggested by others<sup>24,25</sup>. Whichever is correct, we agree with Butler *et al.* that the palaeomagnetic evidence suggests the time breaks in the section measured by them must be relatively short.

Considering the rate of sedimentation of the Scollard Formation to be representative of that of the Edmonton Group (about  $65 \text{ m Myr}^{-1}$ ), one average metre represents about 15,000 yr. A check on the validity of this figure may be made using the approach of Kent<sup>26</sup>. The thickness of the reversed magnetozones between anomalies 29 and 30 in the Scollard Formation is 30 m.

## Tarling and Mitchell

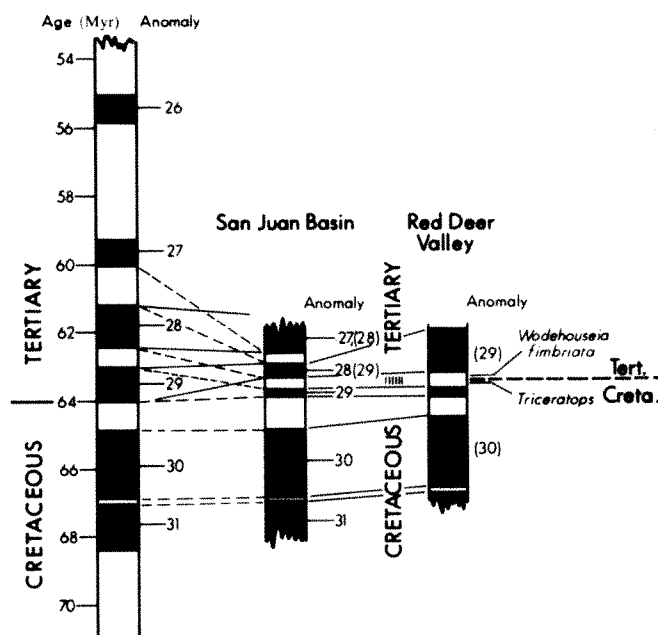


Fig. 3 Suggested correlation of the Red Deer Valley section with the San Juan Basin section and the polarity time scale of Tarling and Mitchell. Dashed tie lines are correlations made by Butler *et al.*<sup>7</sup>. Solid tie lines and anomaly numbers in brackets are correlations suggested in the text. Diagram modified from Butler *et al.*<sup>7</sup>.

This represents a time span of 470,000 yr according to the latest polarity time scale<sup>3</sup>, providing a calculated sedimentation rate of  $63.7 \text{ m Myr}^{-1}$  or  $15,700 \text{ yr m}^{-1}$ . Thus the last dinosaur remains in Alberta were buried about 120,000 yr before the beginning of anomaly 29, and the discrepancy in time between the apparent dinosaur and palynofloral extinctions in this area is about 90,000 yr.

The marine foraminiferal extinctions marking the Cretaceous–Tertiary boundary in the Gubbio section in Italy occur at the base of the *Globigerina eugubina* zone which has been shown to be about 1.5 m below the base of anomaly 29 (ref. 21). Using a mean value of  $6.5 \text{ Myr}$  for the time span of the Maestrichtian<sup>5</sup>, the sedimentation rate for the Maestrichtian part of the Gubbio section is about  $12 \text{ m Myr}^{-1}$ . The rate calculated for the reversed magnetozones between anomalies 29 and 30 is  $12.8 \text{ m Myr}^{-1}$  (ref. 26). Therefore, the time interval between the Cretaceous–Tertiary boundary (based on foraminiferal changes) and the beginning of anomaly 29 was also about 120,000 yr. If our positioning of the base of anomaly 29 in the Red Deer Valley is correct, the foraminiferal Cretaceous–Tertiary boundary and the extinction of the dinosaurs are calculated to be very close in time. Uncertainties in sedimentation rates leave room for some deviation from exact synchronicity. An error in sedimentation rate of as much as 25%, which seems highly unlikely, would produce a maximum difference of about 100,000 yr. Similar studies need to be made on the marine reptilian and ammonite extinctions, but our data indicate approximate synchronicity between at least some marine and continental faunal extinctions at the end of the Cretaceous, by whatever cause.

As the evidence for dinosaur, foraminiferal and floral crises is present just below anomaly 29, it is advocated that the base of anomaly 29 be tentatively accepted as the best global physical approximation of the Cretaceous–Tertiary boundary.

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# Observations of seafloor spreading in Afar during the November 1978 fissure eruption

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*In November 1978 a telluric crisis was recorded in the Republic of Djibouti, in connection with the opening of new distensive fractures in the Asal graben, the southernmost axis of the seafloor spreading in Afar. A short fissure eruption (7–14 November 1978) developed which allowed interesting observations of ridge-type volcanism and gas composition, and accurate measurement of seafloor spreading in Afar for the first time.*

It has been known since the early seventies<sup>1</sup> that the so-called 'Afar triangle' can be divided into two quite different structural units: (1) the Red Sea–Gulf of Aden megastructure (NNW and WNW trends respectively) which belongs to the mid-oceanic ridge type and corresponds to the active boundary between the parting Arabic and African plates, temporarily emerged in Afar; and (2) the northern end of the continental Great Rift Valley of East Africa.

The small Asal graben, approximately 40 km across and 12 km long, located between Asal Lake (155 m below sea-level) and the Gulf of Tadjoura, is the southernmost axis of the present seafloor spreading in Afar<sup>2</sup> (Figs. 1 and 2). The extremely young age of both volcanic products and tectonic manifestations (faults, uplifts) in the Asal graben<sup>3</sup> suggested that a geodimetric network should be set up in that area. The network was positioned by the Institut Géographique National in 1973 over the active axis to measure any increase in distance between the two drifting plates<sup>4</sup>.

## The fissure eruption

On 6 November 1978, a series of more than 800 earthquakes was recorded by the Arta seismographic observatory, some of them reaching a magnitude of 3.3 and an intensity high enough to alarm people in the city of Djibouti (70 km away). The actual eruption started the next day and lasted exactly one week.

A set of two dozen parallel normal faults was observed in a belt more than 1500 m wide in the axial area. Some of the faults reached 10 km long. The general fault direction was 300–320° (NNW), that is, parallel to the Red Sea tectonic trend. No significant lateral shifting was detected. The spacing between fault lips varied from a few tenths of a millimetre to more than a metre. The maximal throw observed was more than 0.5 m. The

whole set constituted a graben the depth and width of which can easily be determined by new geodimetric measurements. The increase in distance between the African and Arabian plates, roughly the sum of each fault width, can be determined exactly.

This event supports the hypothesis that the seafloor spreading mechanism works by sudden jerks, occurring a few times per century, rather than by a continuous smooth distension<sup>5</sup>. Average rates of seafloor spreading in the Asal rift area<sup>5</sup> should be referred to in terms of 1.5 m per century rather than 1.5 cm per yr.

The fissure eruption developed over a 0.75-km long segment of one of the normal faults, directly on the active axis. We called it 'Ardoukoba' (slope down, in Afar language) after the place name<sup>6</sup>. It was exclusively effusive and no actual explosions occurred: the only comparatively violent gas exhausting phenomena were lava fountains, which were generally 20–100 m above lava level. Lava flows spread rapidly over the depression. A lava pool developed, together with the building

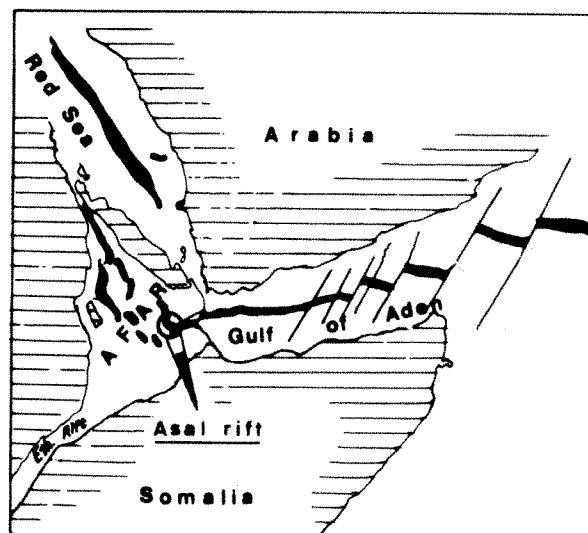


Fig. 1 Location of the Asal rift in the Afar depression. Black areas are zones of generation of new oceanic crust.

**Table 1** Composition of the Ardoukoba gas samples (mol %)

	% Total H <sub>2</sub> O	N <sub>2</sub>	O <sub>2</sub> + Ar	CO <sub>2</sub>	SO <sub>2</sub> (mol % of the 'dry' gases)	H <sub>2</sub> S	CO	H <sub>2</sub>	COS	C/S	CO/CO <sub>2</sub>	p <sub>O<sub>2</sub></sub> (atm)
Precision		±1%	±1%	±1%	±1%	±1%	±10%	±1%	±10%			
A92	62 ± 3	45.0	11.0	8.3	35.3	2 × 10 <sup>-4</sup>	0.15	0.11	10 <sup>-4</sup>	0.24	1.8 × 10 <sup>-2</sup>	10 <sup>-9.43</sup>
A89	59 ± 7	50.9	12.8	6.8	29.3	6 × 10 <sup>-3</sup>	0.11	0.02		0.24	1.6 × 10 <sup>-2</sup>	10 <sup>-9.33</sup>
									to			
A88	ND	47.4	11.2	6.6	34.7	0	0.09	0.02		0.19	1.3 × 10 <sup>-2</sup>	10 <sup>-9.15</sup>
G82	47 ± 7	57.9	15.4	3.8	22.6	3 × 10 <sup>-3</sup>	0.16	0.06	5 × 10 <sup>-4</sup>	0.16	4.2 × 10 <sup>-2</sup>	10 <sup>-10.15</sup>

Analysed by gas chromatography in helium with a catharometer cell. H<sub>2</sub> was detected in argon and H<sub>2</sub>S using a flame ionisation detector. H<sub>2</sub>O was determined by weighing. Measurements were from flask samples from flowing lava at 1,070 °C. ND, not determined.

up of a steep spatter rampart, 40 m high, erected on the fracture's lips as an oblong crater.

For the first few days of the eruption, the boiling lava pool had a nearly 2,000 m<sup>2</sup> surface, which was continuously overturned by the fountaining. The violence of the phenomenon prevented us from reaching the crater lip to sample eruptive gases and we had to wait for the last phase of the eruption before we could do so. A plume, the velocity of which was estimated at 10 m s<sup>-1</sup>, escaped from this crater. From 12 November the lava pool surface was restricted to 400 m<sup>2</sup>. The eruption ended on 14 November with a short, more explosive phase. The total gas output for the duration of the eruption (six effective days) is evaluated as 6 × 10<sup>9</sup> m<sup>3</sup>.

## Sampling

Gas samples were collected on the fracture on 13 November from lavas flowing out of the crater (temperature = 1,070 °C). Severe conditions restricted numbers of sampling to four only. More numerous (but less representative) samples were collected on 14 November from cracks in recent cooling lava flows. Gases were collected in evacuated glass flasks, containing either an alkaline solution or P<sub>2</sub>O<sub>5</sub> dessicator, connected to a 1.5-m long silica pipe. The alkaline-type flask showed the gas to be considerably mixed with air. Therefore, we also used a technique which allows the gas sample to remain in the same dilution as the plume, preserving the gaseous ratios. It was analysed with a high sensitivity (<1 p.p.m. for most gases investigated): the previously dehydrated gases are inspired by a peristaltic pump, stored in a Teflon bag and then immediately analysed by specific reactive 'Dräger' tubes.

Temperature measurements were taken simultaneously using a chromel-alumel thermocouple (±1 °C). Locations, temperatures and gas compositions are reported in Tables 1 and 2.

## Results

Air admixed with the magmatic gas found between the flowing lava and the upper solidified crust through which we sampled. Atmospheric values for N<sub>2</sub>/O<sub>2</sub> + Ar ratios, calculated from the O<sub>2</sub> + Ar contents in the four glass flasks, assuming that all Ar comes from air contamination, yield an excess nitrogen which either corresponds to a true magmatic excess or reflects a partial oxidation of the gas by atmospheric oxygen.

The gases were found to be richer in sulphur than carbon. The C/S atomic ratio (0.2) is lower than that typical of high temperature basaltic gases sampled from central vents (1.5 ± 0.3)<sup>7</sup>. Otherwise, similar C/S ratios have been found in gases sampled from early degassed basalts in Hawaii<sup>8</sup> and particularly from comparable lava flows from Mt Etna<sup>9,10</sup> or the Surtsey eruption (1966–67 samples)<sup>11</sup>. This lower ratio may be typical of a later degassing stage of the lava. Conversely the 14 November gas samples (evacuated flask samples and Teflon bag samples), collected from cooling lavas at sub-solidus temperatures are richer in CO<sub>2</sub> than in SO<sub>2</sub>, a feature more characteristic of gases exsolved from solidifying hot rocks. In these samples H<sub>2</sub>S is also rich relative to SO<sub>2</sub>.

CO/CO<sub>2</sub> ratios are in the range 1.3–4.2 × 10<sup>-2</sup>. Experimental data<sup>12</sup> showed that: (1) the oxidation state of a high-temperature gas phase is consistent with the oxygen fugacity in the melted basalt; (2) the CO/CO<sub>2</sub> ratio is the most reliable redox ratio, compared to H<sub>2</sub>/H<sub>2</sub>O or H<sub>2</sub>S/SO<sub>2</sub>, and is more sensitive to oxidation and to secondary gains or losses caused by condensations, reactions or additions.

Under a pressure of 1 atm and collection temperature (1,343 K) conditions, the oxygen partial pressure in equilibrium with CO and CO<sub>2</sub> can be calculated from:

$$\log p_{O_2} = 2 \left( \frac{\Delta G_T^\circ}{9.150T} - \log \frac{p_{CO}}{p_{CO_2}} \right)$$

where  $\Delta G_T^\circ = -135,330 + 41.74T$  (calculated from standard thermodynamic data<sup>13</sup>).

Table 1 lists the four p<sub>O<sub>2</sub></sub> values obtained, and the mean oxygen partial pressure of the gas is found to be p<sub>O<sub>2</sub></sub> = 10<sup>-9.40</sup> atm. This is in remarkable agreement with the p<sub>O<sub>2</sub></sub> value in the Erta Ale gases (Afar)<sup>12</sup>, and consistent with the oxygen fugacities measured or deduced from mineral assemblages in basalts<sup>14,15</sup>.

Water vapour is generally the major gas present and often amounts to 80–90 mol % of the total, even in higher quality samples collected previously<sup>12</sup>. The interesting point in the present results is the relative deficiency in H<sub>2</sub>O content of the Ardoukoba samples. The measured H<sub>2</sub>/H<sub>2</sub>O ratio (about 10<sup>-3</sup>) is not consistent with the mean p<sub>O<sub>2</sub></sub> calculated above: a p<sub>O<sub>2</sub></sub> of 10<sup>-9.4</sup> atm would lead to an equilibrium H<sub>2</sub>/H<sub>2</sub>O ratio of about 10<sup>-2</sup>. As water vapour clearly condensed inside the flasks during

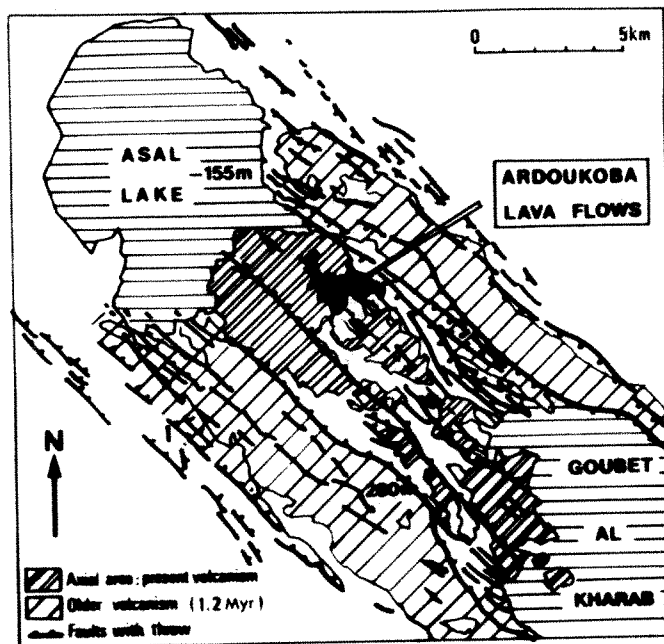


Fig. 2 Position of the Ardoukoba lava flows in the Asal rift.



**Table 2** Composition of gas samples and sublimates from cooling lavas

Precision	CO <sub>2</sub> (p.p.m.) ±5–10%	SO <sub>2</sub> (p.p.m.) ±5–10%	H <sub>2</sub> S (p.p.m.) ±10–15%	HCl (p.p.m.) ±10–15%	C/S	Cl/S	Sublimates (wt %)
W lava flow	2,000	230	5	22	8.5	0.09	SO <sub>4</sub> <sup>2-</sup> 63.07
W lava flow	4,200	850	12	30	4.9	0.03	
W lava flow	4,000	600	11	24	6.5	0.04	Na 24.66
W lava flow	1,100	100	3	9	10.7	0.09	
W lava flow	1,500	120	6	15	11.9	0.12	K 5.83
W lava flow	400	20	0	0	17.4	—	
W lava flow	600	50	1	4	11.8	0.08	Ca 0.48
S spatter	1,200	160	3	9	7.4	0.05	
E lava flow	800	80	2	9	9.7	0.10	Fe 0.61
W base cone	1,400	160	5	10	8.5	0.06	Cu 5.35
Crater rim	2,000	320	6	3	6.0	0.01	
							Cl Mg B <0.1

Sublimates were analysed using an EDS. (Five analyses of green and white sublimates.) Measurements were from Teflon bag samples (810–910 °C).

sampling (temperature at the opened end of the sampling pipe reached more than 400 °C), we can be certain that no water vapour was lost through the flask's stopcock. We suggest two hypotheses to explain this discrepancy: (1) A partial oxidation of the initial H<sub>2</sub> occurs (1 mol % calculated from H<sub>2</sub>/H<sub>2</sub>O = 10<sup>-2</sup>), consistent with the partial deficiency in the O<sub>2</sub> contents when air contamination is calculated on the N<sub>2</sub> basis. (2) A preferential enrichment in H<sub>2</sub>O occurs by continuous adsorption on the dessicator in excess during the few seconds the flasks were open. If this were so, the equilibrium H<sub>2</sub>O contents might have been about five times less than they were actually found to be in the samples.

At present it is not possible to decide between these two hypothetical mechanisms, and they may have acted together. Also, the H<sub>2</sub>O content of the samples has to be considered as an upper limit to the water vapour fraction of these eruptive gases which therefore appear particularly anhydrous.

The HCl content in samples from cooling lavas (Table 2) is surprisingly low as it has been shown from many volcanoes that similar gases from early degassed lavas are generally more chloride-rich than those from central vents. Atomic Cl/S ratios in Ardoukoba gases are still lower than Cl/S ratios in gases collected from the Erta Ale lava lake (0.17) or from lava flows of the oceanic Surtsey eruption (0.03–0.5)<sup>11</sup>.

From the proximity of highly saline waters in Asal Lake (NaCl > 350 g l<sup>-1</sup>), 2 km away, and of seawater in the Ghoubet, 10 km away (Fig. 2), one could have expected a marine type

contamination of the magma and gases, the eruption point of which was situated about 100 m below sea level.

In fact, the collected samples appear rather lacking in vapour and chloride poor. Moreover, microprobe energy dispersion spectrometer (EDS) analyses of incrustation deposits from around the fuming points (Table 2) show no anomalous sodium enrichment and show also that these sublimates are particularly depleted in chloride, boron and magnesium.

The total lavas welled out by the eruption amounts to 16 × 10<sup>6</sup> m<sup>3</sup>, the average thickness of the 1.6 km<sup>2</sup> lava field being estimated at about 10 m. This highly fluid lava contains a high proportion of phenocrysts, mainly large crystals of plagioclase (bytownite) up to 4 cm long, and far less olivine and clinopyroxene. It is identical to the particularly plagioclase-rich basalts widely outcropping in the active part of the Asal graben<sup>5,16</sup> (Table 3).

## Discussion

Note that this short, but volumetrically important, eruption is similar to numerous recent ones, the evidence of which we observed in this area and throughout the whole northern and central Afar depression, and is probably representative of most of the fissure eruptions in rift areas. It is interesting as a reference for the so many, but invisible, submarine fissure eruptions. In particular, it is useful to compare the ratio of the massic gas and lava outputs with the volatile amounts trapped in fresh sub-oceanic basalts. 16 × 10<sup>6</sup> m<sup>3</sup> of solidified basalt with a density of 2.7 g cm<sup>-3</sup> yields 43 × 10<sup>6</sup> tons of lava discharged by the Ardoukoba eruption, and the mass of 6 × 10<sup>9</sup> m<sup>3</sup> of emitted gas, the density of which could be considered to be ~0.25 g l<sup>-1</sup> at 1 atm and 1,100 °C, is 1.5 × 10<sup>6</sup> tons. Then, the mass gas output amounts for 3% of the lava production. This is rather small, when compared with the recently measured gas discharge from other volcanoes<sup>17</sup>, which widely exceeds the initial volatile content the corresponding erupted magma could supply.

This leads us to consider that the magmatic reservoir from which the Ardoukoba eruption developed may have been a closed system. The relative 3% massic gas to lava output is scarcely a few times the volatile content in sub-oceanic ridge basalts<sup>18</sup>.

This low gaseous output, the particularly low water vapour content, the apparent lack of marine contamination which might have been expected, the short duration of the eruption and the highly crystallised state of the produced lava, make us think that the magma intrusion which supplied the Ardoukoba eruption was quite narrow and probably close to the surface, and hence reduced the disturbance the eruption could have brought about in the geological surroundings.

**Table 3** Chemical composition (wt % oxides) of the Ardoukoba lava and phenocrysts

	Lava flow	Pyroclastite	Olivine	Pyroxene	Plagioclase
SiO <sub>2</sub>	48.01	48.01	37.55	51.22	46.55
TiO <sub>2</sub>	1.21	1.09	—	0.2	—
Al <sub>2</sub> O <sub>3</sub>	20.72	20.43	—	1.4	33.91
FeO + Fe <sub>2</sub> O <sub>3</sub> *	8.90	8.59	—	—	—
FeO	—	—	15.36	4.26	0.21
MnO	<0.1	<0.1	0.13	—	—
MgO	3.50	3.99	46.82	17.91	—
CaO	15.26	15.46	0.14	22.44	17.90
Na <sub>2</sub> O	2.35	2.29	—	—	1.43
K <sub>2</sub> O	<0.05	<0.05	—	—	—
P <sub>2</sub> P <sub>5</sub>	<0.1	<0.1	—	—	—
Cr <sub>2</sub> O <sub>3</sub>	—	—	—	0.33	—
Total	100.2	100.1	100	97.8	100

Analyses were carried out using an energy dispersion spectrometer

\* Based on equimolar ratios.

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# Fluorine in Iceland and Reykjanes Ridge basalts

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*Unlike chlorine and bromine, fluorine is not appreciably outgassed from basalts erupted above 500 m depth along the Reykjanes Ridge and subaerially over Iceland. The mantle beneath Iceland seems to be twice as rich in fluorine as the asthenosphere source of normal ridge basalts south of 61°N.*

OCEANS and atmosphere are generally accepted to have formed over geological time by the outgassing of volatiles from the Earth's interior during volcanism<sup>1</sup>. But very little is known about the abundance, distribution and relative fractionation of volatiles in the mantle. Nor do we fully understand the nature of the processes of transport and release of volatiles to the Earth's surface. The study of volatiles in lavas derived from the mantle is complicated by the tendency of such elements to be partly lost during the ascent of magmas, either before erupting at shallow depth under the sea or during subaerial eruption. Furthermore, volcanic gases collected near volcanic vents may not be juvenile, but partly or totally derived from fluids of meteoric origin. Thus, knowledge of the geochemical evolution of volatiles in the mantle has lagged behind that of the more refractory elements. The study of submarine basalts and basaltic glasses, pioneered by Moore<sup>2</sup>, has opened up new opportunities for the investigation of volatiles.

Unni and Schilling<sup>3</sup> have described the distribution of chlorine and bromine in basalts from the Reykjanes Ridge and Iceland, contrasting subaerial with submarine volcanism as affected by hydrostatic pressure. They found that both chlorine and bromine were drastically fluxed out from lavas erupted at depths of less than 500 m below sea level (approximately 50 bar hydrostatic pressure), coincident with the depth of extensive vesiculation of water observed in several instances<sup>2,4–8</sup>. Chlorine and bromine degassing occurs although neither of the two halogens is saturated in the silicate melt in the pressure-temperature conditions of eruption<sup>3</sup>. Fluxing of sulphur and selenium has also been observed at this depth<sup>7,8</sup>. Unni and Schilling<sup>3</sup> have considered the possibility of fluxing of chlorine and bromine as due to a strong tendency for these two volatiles to separate into the aqueous fluid phase relative to the melt during vesiculation, in accordance with the experimental partition data of Kilinc and Burnham<sup>9</sup>. However, the amount of water actually exsolving seemed to be insufficient, and CO<sub>2</sub> (and/or SO<sub>2</sub>) was invoked as an additional possible major flux carrier for outgassing chlorine and bromine.

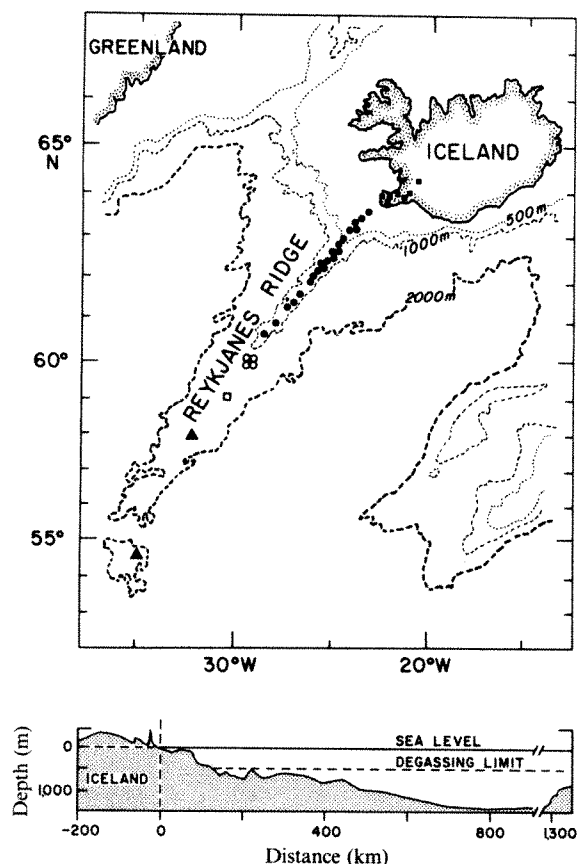
Fluorine, although another member of the chemically related halogen group, might be expected to behave differently in a silicate melt-aqueous fluid system. Its smaller ionic radius, 1.34 Å compared with 1.81 Å for chlorine and 1.96 Å for bromine, permits fluorine to substitute easily for OH<sup>–</sup> in hydrous minerals or melts<sup>10</sup>. The few experimental data available for partitioning of fluorine and chlorine between silicate melts and a co-existing H<sub>2</sub>O fluid phase at high temperature and pressure<sup>9,11</sup> suggest partition coefficients of 3:1 for fluorine in favour of the melt, but less than 1:30 for chlorine. The existing data are for granitic-type melts. As far as we know there is no corresponding data for melts of tholeiitic composition.

We report here the fluorine content of basalts from the Reykjanes Ridge-Iceland profile previously analysed by Unni and Schilling<sup>3</sup> for chlorine and bromine. This part of the Mid-Atlantic Ridge, as it shoals from 1,380 m to its subaerial expression in the south-west neo-volcanic zone of Iceland, affords an excellent opportunity to study the behaviour of volatile elements as a function of hydrostatic pressure. Locations and depths from which the samples were dredged are shown in Fig. 1. This area also spans from rare earth<sup>12</sup> and isotope data<sup>13,14</sup> two apparently separate and distinct mantle sources of magma with a zone of mixing between them. Furthermore, it has been shown<sup>3</sup> that chlorine and bromine variations along the Reykjanes Ridge-Iceland profile fit quite well a predictive model compounded from a curve of the distribution of large ion lithophilic trace elements (LILE) from two different adjacent mantle sources and their mixing, and a curve representing the degassing of a volatile from lavas derived from a single homogeneous mantle source as a function of decreasing hydrostatic pressure above 500 m of depth. The abundance of fluorine in the same basalt samples should not only reveal the distribution of fluorine along the Reykjanes Ridge and its extension beneath Iceland, but also yield useful information relevant to degassing mechanisms of the three halogens and their relative fractionation during degassing.

## The fluorine profile

Fluorine was determined by a specific ion electrode procedure adapted from the method of Ingram<sup>15</sup> for fluoride in silicate rocks, combined with the procedure of Huang and Johns<sup>16</sup> for fusion and preparation of sample solutions. The method was standardised against the literature values of Flanagan<sup>17</sup> for the rock standards AGV-1 and JB-1, and was reproducible to ±15 p.p.m. in the relevant concentration range. The samples are fresh basalts dredged from the axis of the ridge<sup>12</sup>, and analyses

were carried out on powders prepared for the most part from the variolitic zone of pillows, and a few from glasses from pillow rims. A summary of individual fluorine concentrations in pillow basalts from the Reykjanes Ridge and Iceland is given in Table 1, where they are compared with the chlorine values of Unni and Schilling<sup>3</sup> determined on the same samples.



**Fig. 1** The Reykjanes Ridge and Iceland, showing depth contours and sample locations. Cruise numbers are: ●, TR 101; ○, TR 41; □, GLJ; and △, TR 100; and subaerial samples are designated by ■.

The variation of fluorine content with latitude (Fig. 2) shows a progressive increase of fluorine towards Iceland. The fluorine profile resembles those of refractory incompatible trace element ratios such as  $[La/Sm]_{EF}$  (Fig. 2a), or of isotope ratios<sup>13,14</sup> of  $^{87}Sr/^{86}Sr$  or  $^{206}Pb/^{204}Pb$  and  $^{208}Pb/^{204}Pb$ . Furthermore, fluorine variation does not seem to be related in any simple way to Al, Mg, or Ca variation in these basalts along the Reykjanes Ridge–Iceland profile. Nor is it related to silica content, which remains constant at  $50 \pm 0.3\%$  in submarine samples and drops to 47–49% over Iceland (data not shown). Indices of major element fractionation such as  $\Sigma Fe/\Sigma Fe + Mg$  and  $Na_2O/CaO$  pass through a maximum over the Iceland shelf coincidental with the en echelon displacement of the ridge axis<sup>18,19</sup>, whereas fluorine continues increasing towards Iceland. These observations rule out the possibility of explaining the fluorine increase towards Iceland as being due to increasing fractional crystallisation or decreasing degrees of partial melting of a single mantle source toward Iceland (see for example refs 12, 20–22 for discussions on the subject).

In marked contrast to the other volatiles Cl, Br, S and Se, fluorine does not show any appreciable degassing above the apparent 500 m critical depth of extensive vesiculation of water vapour observed in several instances<sup>2,3,5,7,8</sup> (Fig. 2). Although fluorine follows the same trend as chlorine up to 63°N at depths

greater than 500 m, the fluorine content of basalts in the northernmost section of the profile remains high while chlorine decreases due to progressive degassing with decreasing depth of eruption<sup>3</sup>. The failure of fluorine to degas is further emphasised in Fig. 3, which compares variation of fluorine with variation in lanthanum, a refractory element; potassium, a less refractory element; and chlorine, a volatile element. For both lanthanum versus fluorine and potassium versus fluorine the Iceland field (subaerial) overlaps with the trend for the Reykjanes Ridge. But in the case of chlorine versus fluorine, the Icelandic field has moved down along the chlorine axis and is quite distinct from the Reykjanes Ridge basalts, because of the loss of chlorine during subaerial eruption<sup>3</sup>.

The relative fractionation of fluorine with respect to chlorine in the Reykjanes Ridge basalts is shown in Fig. 2, using Cl/F ratios from Table 1. The ratio increases on approaching land, apparently due to enrichment of the larger ion in the Iceland mantle source compared with the LILE-depleted asthenosphere. But at depths less than 500 m and in subaerial samples the ratio drops drastically because of the loss of chlorine with concurrent water vesiculation. From a maximum value of 1:1 the Cl/F ratio drops to about 0.1, or by approximately an order of magnitude.

**Table 1** Fluorine content of Iceland and Reykjanes Ridge basalts (pillow interiors)\*

Station	Lat (N)	Long (W)	Distance† (km)	Depth (m)	F (p.p.m.)	Cl‡ (p.p.m.)	Cl/F
<b>Iceland</b>							
IC-17	64°47.2'	20°42.7'	-139	+400	248	45	0.18
IC-11	64°16.75'	21°07'	-64	+150	211	24	0.11
IC-36	64°12.6'	20°01.2'	-59	+250	155	30	0.19
IC-57	63°55.8'	22°26.5'	-29	+72	270	99	0.37
IC-62	63°58.7'	21°58'	-23	+140	193	60	0.31
IC-47	63°58'	21°44.5'	-22	+460	214	64	0.30
IC-58	63°54.8'	22°26'	-14	+70	248	93	0.38
<b>TR 101</b>							
15D-13A	63°34.7'	23°42.2'	33	33	233	128	0.55
18D-1	63°28.1'	23°51'	49	43	251	172	0.68
11D-2	63°16.3'	24°12.4'	78	75	230	173	0.75
12D-7	63°12.9'	24°16'	86	258	270	199	0.73
14D-5C	63°11.2'	24°27.6'	91	345	261	232	0.89
6D-9	62°59.7'	24°41.9'	118	400	222	170	0.76
3D-1A	62°47.5'	25°09.6'	147	620	179	152	0.85
35D-3A	62°42'	25°12.3'	160	580	176	185	1.05
2D-1,2	62°37.4'	25°26'	172	633	217	226	1.09
1D-2	62°35.6'	25°27.5'	176	618	206	211	1.02
22D-1	62°22.4'	25°50.7'	208	715	153	166	1.08
23D-1	62°21.3'	25°36.8'	210	655	169	116	0.70
34D-6	62°16.1'	26°08.4'	221	500	151	96	0.64
24D-6C	62°05'	26°17.8'	248	692	182	51	0.28
27D-1	61°44'	26°52.9'	298	600	120	140	1.17
29D-5A	61°05.9'	27°52.7'	390	810	86	85	0.99
30D-10A	61°05.9'	27°54.2'	390	792	82	65	0.79
31D-9C	60°44'	28°54.2'	440	710	86	16	0.19
33D-6A,B	60°27.4'	28°53.1'	482	923	137	36	0.26
<b>TR 41</b>							
D46-1	61°22'	27°24'	352	650	124	67	0.54
D22-1	60°01.2'	29°28.5'	543	978	126	64	0.51
D20-3	60°01.2'	29°20.8'	543	948	254	108	0.42
D18-2	59°59.7'	29°32.5'	545	1040	99	62	0.63
D38-2	59°59.1'	29°31.5'	547	925	132	71	0.54
GLJ-10	58°52.1'	30°56.7'	705	1383	98	70	0.71
<b>TR 100</b>							
26D-10	57°41'	32°34'	885	1380	180	69	0.43
23D-10	54°15'	35°24'	1340	880	127	28	0.22

\* Our precision for F analyses of standards AGV-1 and JB-1 were  $439 \pm 19(1\sigma)$  and  $365 \pm 9(1\sigma)$ , respectively. Corresponding recommended values are  $435 \pm 20$  and 360 (ref. 17).

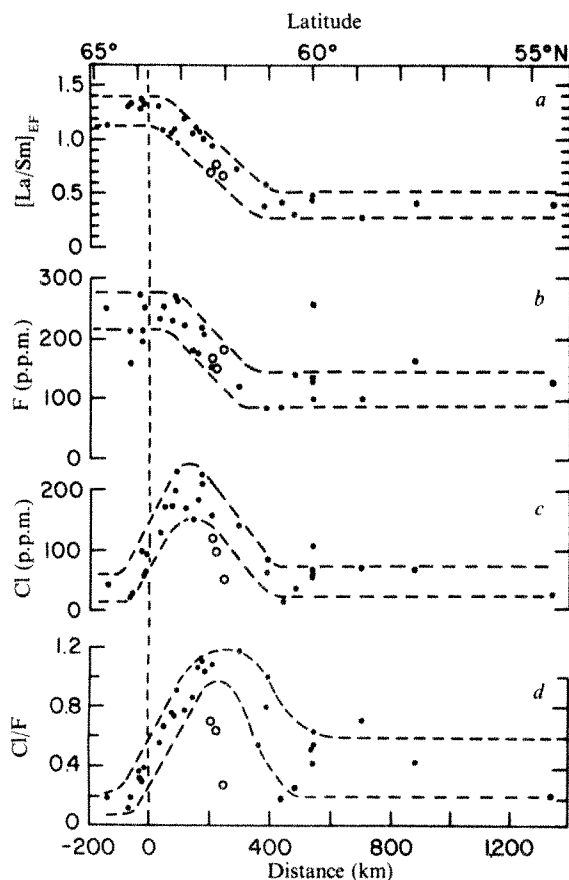
† Distance SW along the Reykjanes Ridge Axis; taken equal to zero at the SW tip of the Reykjanes Peninsula.

‡ Cl values from Unni (ref. 46).

Yet, despite the evidence suggesting absence of degassing of fluorine from the Reykjanes Ridge basalts, fluorine is often found to be present in volcanic gases and emanations.



Measurements of the F/Cl ratio in gases from Hawaiian volcanoes range from 0.3<sup>23</sup> to 0.003<sup>24</sup>. Moreover the incidence of fluorine poisoning in grazing livestock following a major Icelandic eruption has been directly correlated with wind direction, distance from the eruption vent, and thickness of tephra layers<sup>25</sup>. Thorarinnsson<sup>26</sup> describes measurements of 350 p.p.m. fluorine in ash particles less than 0.06 mm in diameter compared with 50 p.p.m. in an 0.25 mm fraction, such enhancement has been interpreted<sup>27</sup> as indicating adsorption of gaseous HF on the surface of the ash during its eruption. Thorarinnsson<sup>26</sup> has estimated that at least  $3 \times 10^4$  tons of fluorine were discharged with the tephra of the Mt Hekla eruption in 1970.



**Fig. 2** Comparison of the F profile (b) with the La/Sm enrichment factor ratio (a), Cl (c), and the Cl/F ratio (d).  $\circ$  are anomalously low in Cl (and also Br) but not in F or refractory elements. Morphologically, they are rounded vesicular basalts (not pillows), and represent probably lava erupted subaerially, subsequently eroded by wave action during subsidence (or submergence). The low Cl and Br, but not F nor LILE contents, seem to corroborate our observations made previously on ship.

Our evidence for the lack of any substantial fluorine degassing at eruption depths of less than 500 m along the Reykjanes Ridge would seem paradoxical. This is not the case. A simple calculation using the Rayleigh distillation equation<sup>28</sup> predicts that, if a maximum of 0.5–1.0% H<sub>2</sub>O vapour exsolved, the chlorine and fluorine concentrations of the vapour would be of the order of 5,000 p.p.m. and 70 p.p.m. (assuming vapour–melt partition coefficients of 30 and 0.33) respectively. The F/Cl ratio in the vapour would be 0.014, thus well within the range observed on land<sup>23,24</sup>. The fluorine content of the remaining magma would have increased only by 1 p.p.m. maximum, which is well within the analytical uncertainties.

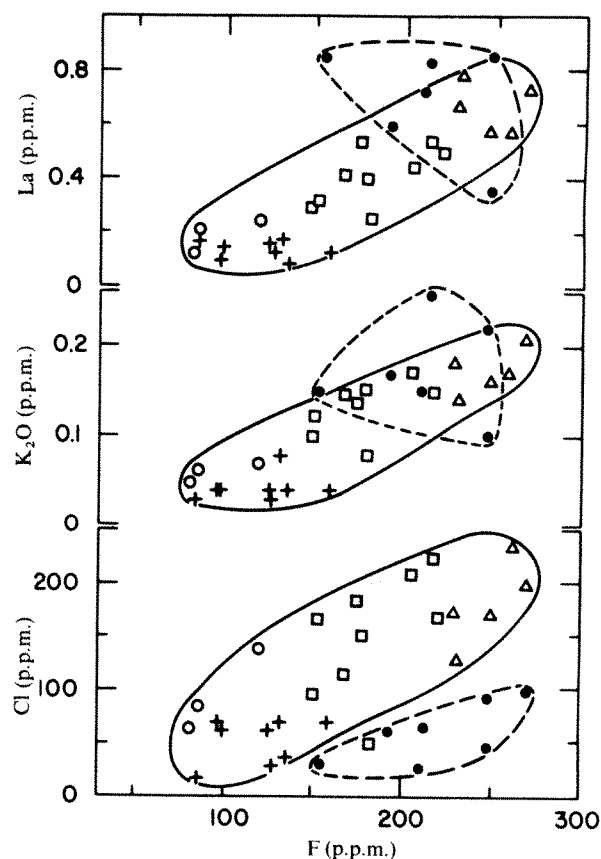
The lack of appreciable outgassing of fluorine along the ridge may to some extent be further tested by comparing variation in fluorine with variation in a refractory lithophilic element which might vary coherently with fluorine. Data from the area of the Mid-Atlantic Ridge encompassing the Azores platform, a section well below the 500 m critical depth of degassing, indicate that fluorine and strontium, or fluorine and phosphorous do not fractionate appreciably relative to each other during partial melting or fractional crystallisation<sup>29,30</sup>. The two ratios remain constant despite clear evidence from <sup>87</sup>Sr/<sup>86</sup>Sr data<sup>31,32</sup> of a change in mantle source beneath the Azores. Figure 4 shows variation of the F/Sr and F/P ratios with distance along the Reykjanes Ridge–Iceland profile.

Equations for least-squares best-fit slopes are:

$$F/P = 3.28 \times 10^{-5} d + 0.289$$

$$F/Sr = 1.22 \times 10^{-4} d + 1.75$$

where  $d$ , the distance in km south-west along the Reykjanes Ridge axis, is set equal to zero at the south-west tip of the Reykjanes Peninsula on Iceland. In other words, both ratios remain essentially constant along the profile. The subaerial data are insufficient to establish any significant loss of fluorine. Even if the sample IC-17 (anomalously high in fluorine content) is not considered, the small drop in F/Sr and F/P ratios over Iceland would suggest a maximum loss of only 5–10%. We conclude from this and from the Cl/F ratio profile that unlike chlorine and bromine, fluorine does not appreciably degas from lava erupted subaerially or above the 500 m depth. A comparison of the maximum and minimum Cl/F ratios would indicate a relative



**Fig. 3** Comparison of F variation with La (refractory), K (less refractory), and Cl (volatile). —, Reykjanes Ridge submarine samples; ---, Icelandic subaerial field.  $\bullet$ , Iceland;  $\Delta$ , 63–64° N;  $\square$ , 62–63° N;  $\circ$ , 61–62° N; +, S of 61°.

fractionation of these two elements of at least a factor of 10 in the shallow water and subaerial basalts.

Yet, the Rayleigh distillation calculation previously discussed predicts only a 25% drop in the Cl/F ratio due to vesiculation of a maximum of 0.5–1% water vapour. The Cl/F ratio could be further reduced to 33% of the maximum observed, if up to 50% of the melt underwent shallow depth fractional crystallisation at the same time as vesiculation occurred. A fluorine bulk crystal–melt partition coefficient of 0.02 was used in the calculation<sup>30</sup>. Note also that these calculations are based on fluid–melt partition coefficients for granitic melts<sup>9,11</sup>. Unless in the case of basaltic melts such vapour–melt partition coefficients are significantly reduced for fluorine and increased for chlorine, vesiculation of a major gas phase other than H<sub>2</sub>O, such as CO<sub>2</sub>, would have to be invoked to account for the major Cl/F drop at less than 500 m depths, as suggested by Unni and Schilling<sup>3</sup> for chlorine alone. A corollary of the chlorine outgassing mechanism proposed for lava erupted above 500 m depth or subaerially is that emanated volcanic gas counterparts should have a Cl/F greater than unity. This seems to be corroborated by the Cl/F ratios of volcanic gases, which though quite variable, are usually greater than unity, and often greater than 10 (refs 23, 24, 33, 34). The preferential affinity of fluorine for the melt compared with chlorine might suggest a different structural model for the solubility of fluorine as opposed to chlorine in silicate melts, although Burnham's<sup>35</sup> solubility model would not support this.

### Fluorine content of the two mantle sources

As previously inferred, the fluorine distribution in the Reykjanes Ridge–Iceland basalts is consistent with Schilling's model<sup>12</sup> of two mantle sources for this region, one depleted in LILE and one more enriched, with an intermediate zone of mixing between them. The fluorine content in the section between 54° and 61°N (deep sea) averages  $114 \pm 24$  p.p.m. ( $1\sigma$ ), which may be taken as characteristic of mid-oceanic basalts derived from a LILE-depleted asthenospheric source. North of 63°N and including seven Icelandic tholeiites from the southwestern neo-volcanic zone, fluorine averages  $232 \pm 32$  p.p.m. ( $1\sigma$ ), with more scatter in subaerial than submarine samples. Fluorine between 61° and 63°N grades between the two levels in a manner grossly consistent with the mixing of two sources. These results suggest a 2–3 fold relative enrichment of fluorine in the mantle source of basalts from the Iceland platform over the source of the ridge south of 61°N.

An estimate of the fluorine content of the two mantle sources of this region may be made by assuming: (1) partial melting of 25–30% over the entire area to produce the magmas, and (2) a reasonable partition coefficient for fluorine between solid and melt during partial melting. Estimates of a fluorine partition coefficient can be made in two ways, considering that no experimental values are available. First, fluorine varies nearly coherently with strontium in the processes of partial melting and fractional crystallisation<sup>29,30</sup>. The strontium partition coefficient between basaltic groundmass and a number of minerals present as phenocrysts measured by Philpotts and Schnetzler<sup>36</sup> varies greatly, depending on the nature of the phenocrysts. Assuming that the two mantle sources under consideration were originally composed of a lherzolite containing 60% olivine, 20% orthopyroxene and 20% clinopyroxene, the bulk partition coefficient for strontium (and hence also F) between such an assemblage and the melt ( $D_{Sr}$ ) would be about 0.04 ( $D_F = D_{Sr} = 0.60 \times 0.01 + 0.20 \times 0.1 + 0.20 \times 0.06$ ).

Second, although the mineral phases of the mantle are speculative, judging from the fluorine value determined by Huang and Johns<sup>16</sup> for the geochemical peridotite standard PCC-1, a fluorine value of 13 p.p.m. would be anticipated for a residual peridotite which has been depleted in LILE by partial melting. Since  $D$  is defined as  $C_R/C_L$ , where  $C_R$  is the concentration in the solid residue after partial melting and  $C_L$  the concentration

in the melt, then, assuming 13 p.p.m. for  $C_R$  and a minimum of 100 p.p.m. F for  $C_L$  in the melt, a partition coefficient ( $D_F$ ) of a maximum of 0.13 would be obtained, which is not far from the partition coefficient for fluorine calculated from a lherzolite mantle model using strontium as an analogue.

The use of a reasonable partition coefficient of 0.06 for fluorine would suggest a fluorine content of  $74 \pm 10$  p.p.m. for the Iceland mantle source, and  $36 \pm 9$  for the LILE-depleted asthenosphere, assuming a degree of melting of 25–30% and using the partial melting equation of Schilling and Winchester<sup>37</sup>.

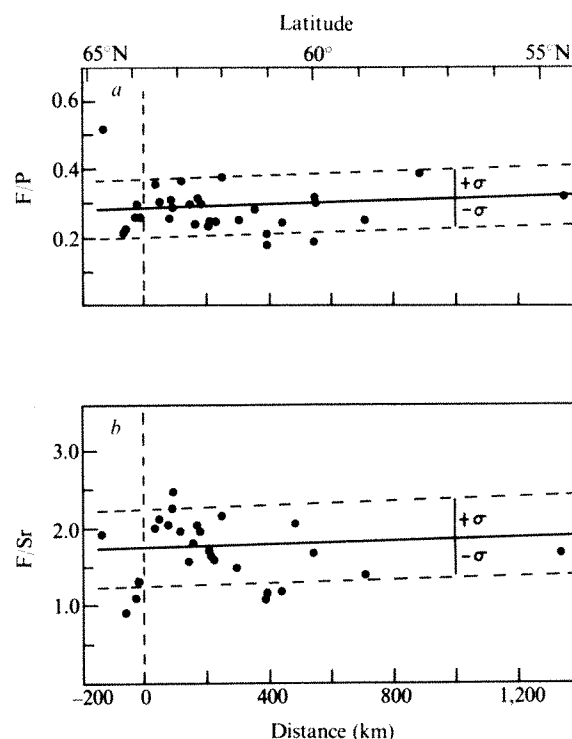


Fig. 4 Variation of F relative to a, P; and b, Sr along the Reykjanes Ridge–Iceland profile. See text for equation of line by linear regression.

### Fluorine in basaltic glasses

We have also examined the possibility of fluoride exchange with seawater, either during submarine basalt formation at the time of quenching, during subsequent cooling of the pillow lava, during low temperature weathering, or during hydrothermal circulation. For this purpose the fluorine content of fresh glasses, glasses altered to various degrees, and a palagonite were compared with pillow interiors (mostly sampled within the variolitic zones). The samples were either from the same basalt pillow or from other pillow fragments recovered from the same station.

Table 2 summarises our results for fluorine in 15 glasses and a sample of palagonite removed from the glassy rim of a pillow basalt from dredge haul TR101-27D, and compares these concentrations with fluorine values of pillow interiors from Table 1. Five of the 15 glasses matched rock fluorine within analytical error and two more were only slightly higher. The others were enhanced in fluorine by a factor of 1.3–3.0, and the enrichment is not apparently related to depth of eruption, latitude or the quantity of fluorine in the corresponding crystalline samples. In no case was fluorine in glass significantly lower than in the more crystalline pillow interior. Several glass samples from one station showed highly variable enrichment in fluorine

content (TR101-27D). A similar increase in content in quenched glassy rims over more crystalline interiors has been observed for several volatiles other than fluorine, such as sulphur<sup>38</sup>, <sup>40</sup>Ar (ref. 39), and H<sub>2</sub>O (ref. 5).

**Table 2** Fluorine content in Reykjanes Ridge basalts (glass rims)

Station	Distance* (km)	Depth (m)	$F_{\text{glass}}$ (p.p.m.)	$F_{\text{rock}}^{\dagger}$ (p.p.m.)	$F_g/F_r$
TR 101					
11D-2G	78	75	458	230	1.99
11D-s.g.†			344	(230)§	1.49
35D-2g	160	580	172	176	0.97
35D-3g			236	(176)	1.34
1D-10g	176	618	230	(206)	1.12
22D-2g	208	715	154	(153)	1.01
27D-1g	298	600	172	120	1.43
27D-13g			364	(120)	3.03
27D-glass			160	(120)	1.33
27D-palagonite			436	(120)	3.63
30D-2g	390	793	202	(82)	2.46
30D-12g			232	(82)	2.83
TR 41					
D22-s.g.	543	978	142	(126)	1.16
D20-s.g.	543	948	251	(254)	0.99
D18-s.g.	545	1040	93	(99)	0.94
D36-s.g.	547	925	138	(132)	1.05
Average					$1.54 \pm 0.70(1\sigma)$

Samples were collected with RV Trident during cruise TR 41 and TR 101.

\* See Table 1.

† Pillow interior (mostly variolitic zone).

‡ s.g., Station glass (loose glass found in the dredge haul).

§ Rock values in parentheses are from a different pillow within the same station.

The cause of fluorine enhancement in some of these glasses is uncertain. There are several possibilities: (1) Incorporation of fluorine from seawater either during quenching or later by low-temperature weathering, would seem unlikely. The fluorine content of seawater is only 1.3 p.p.m. (ref. 40) while the content in pillow interiors is 82–270 p.p.m. Equilibrium of the glass with seawater would tend to reduce fluorine in the glass, if the partition coefficient previously discussed were to apply. (2) Entrapment of bubbles rising from the melt, as proposed by Moore and Schilling<sup>7</sup> for sulphur enhancement in glasses, might be considered. However, in the case of fluorine this explanation also seems unlikely, as first, we have shown that fluorine is not appreciably degassed from vesiculating lava and hence is not mobilised during vesiculation (see the previous calculation on distillation of fluorine), and second, the observed fluorine enhancement was not related to hydrostatic pressure. (3) Fluorine could have been lost from pillow interiors, rather than enriched in the glassy rims. The mechanism of Corliss<sup>41</sup>, by which incompatible elements are concentrated in residual interstitial liquid in a crystallising melt—to be subsequently removed by hydrothermal waters circulating through micro-cracks and fissures, is supported by finding fluorine as one of the elements enhanced in interstitial melts from the Alae Lava Lake of Hawaii<sup>42</sup>. Fluorine data on hydrothermal solutions such as found in the Galapagos Region<sup>43</sup> could be a test of this possibility, when available. Clearly, more information is needed to resolve the question of high glass/rock ratios for fluorine.

## Conclusions

Fluorine does not outgas like chlorine and bromine from submarine lavas erupted at a depth of less than 500 m, the depth of rapidly increasing vesiculation. While fluxing by vesicle-forming major volatiles, such as H<sub>2</sub>O and/or CO<sub>2</sub>, seems to explain the outgassing of chlorine and bromine at shallow depth, such a mechanism is ineffective in removing appreciable quantities of fluorine. Our observations of outgassing of halogens and relative fractionation of chlorine and fluorine during intense vesiculation

at depth shallower than 500 m are also consistent with experimental partition data for the relative distribution of fluorine and chlorine between silicate melts and a co-existing aqueous phase. The proposed outgassing model is also consistent with the contrasting Cl/F ratios less than unity in outgassed lavas and greater than unity in volcanic gas counterparts usually reported. The contrasting outgassing behaviour of fluorine and chlorine (and bromine) emphasises the need of establishing whether a distinct structural model for the solubility of fluorine in silicate melts as compared with chlorine and bromine is required.

The distribution trend of fluorine in basalts from the Reykjanes Ridge and Iceland profile is consistent with the hypothesis of two mantle sources and their mixing for the origin of basalts of this area. The mantle source of the Iceland basalts seems to be enriched by approximately a factor of two over the source of the ridge basalts erupted south of 61°N.

An explanation for our finding of fluorine enrichment in some glass samples over more crystalline pillow interiors (variolitic zone) will require further information.

O'Hara<sup>44</sup> has considered extensive and long-lived contamination of Icelandic magma chambers by previously erupted basalts which may have been hydrothermally altered by circulating seawater. We cannot comment on this mechanism from our fluorine data which does not include hydrothermally altered basalts. However, we note that the <sup>207</sup>Pb/<sup>204</sup>Pb versus <sup>206</sup>Pb/<sup>204</sup>Pb trend previously observed for the Iceland-Reykjanes Ridge profile<sup>14</sup> does not point towards estimated lead isotope ratios for seawater<sup>45</sup>, as would be expected if the O'Hara model were to be applicable to this region.

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# t-Haplotypes of the mouse may involve a change in intercalary DNA

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*The naturally occurring t-haplotypes of the mouse exhibit a set of peculiar genetic properties, including strong suppression of crossing over in the segment of chromosome 17 between the loci of T and H-2. Study of the genetics of mutant haplotypes suggests that the observed effects on meiosis and embryonic development may be due to an altered form of intercalary DNA (iDNA) in the relevant chromosomal region (band 17B).*

THE t-haplotypes of the mouse<sup>1-4</sup> occur commonly in the wild and thus must be considered part of the usual mouse genome. Furthermore, they are located near to the major histocompatibility complex (H-2 complex) on chromosome 17, thereby inviting speculation concerning any possible evolutionary or other genetic significance of this close association<sup>5,6</sup>. Their properties include homozygous lethality and various effects when heterozygous, including enhancement of the effect of the mutant gene for brachyury, T, sterility or segregation distortion in males, and strong crossover suppression in chromosome 17.

The nature of the genetic change in t-haplotypes remains unknown. They are thought to involve some kind of chromosomal changes, but there is no evidence for major structural rearrangements such as inversions<sup>7</sup>, and no indication that rDNA is involved<sup>8</sup>. We suggest here that t-haplotypes involve a change in moderately repetitive DNA (iDNA) intercalated between the structural genes in the segment of chromosome 17 occupied by the haplotypes, extending approximately from the locus of brachyury, T, to the H-2 complex (Fig. 1). This suggestion arises from a study of the retention, loss or alteration of the various properties in 'mutant' t-haplotypes which have been derived from naturally occurring ones by crossing over or other means, and we regard it as a development of our earlier suggestions that t-haplotypes involved 'heterochromatin' (ref. 9) or changes in interstitial heterochromatin<sup>10</sup>.

Heterozygotes for brachyury T typically have short tails, but t-haplotypes interact with T, so that T/t heterozygotes are tailless. When homozygous, naturally occurring t's are lethal or semi-lethal during embryogeny, and fall into six lethal complementation groups, with partial complementation between groups in terms of viability<sup>1</sup>. Surviving t<sup>x</sup>/t<sup>y</sup> males (where t<sup>x</sup> and t<sup>y</sup> are from different groups), and also males homozygous for semi-lethals, are sterile. Furthermore, males heterozygous for a single t-haplotype, that is, +/t or T/t, although fertile, transmit t to the offspring with an abnormal frequency, usually having a large excess of t-carrying young. In addition, natural t-haplotypes strongly suppress crossing over in both sexes in the segment of chromosome 17 extending from T to H-2.

A further characteristic is that of mutating to other forms, with loss or occasionally gain of one or more of the other properties. This 'mutation' is usually, but not always, accompanied by crossing over within the region of strong crossover suppression. Correlation of the genetic properties of the various mutant haplotypes with the apparent position at which the crossover occurred has made it possible to map factors underlying the various properties. The most extensively studied batch of

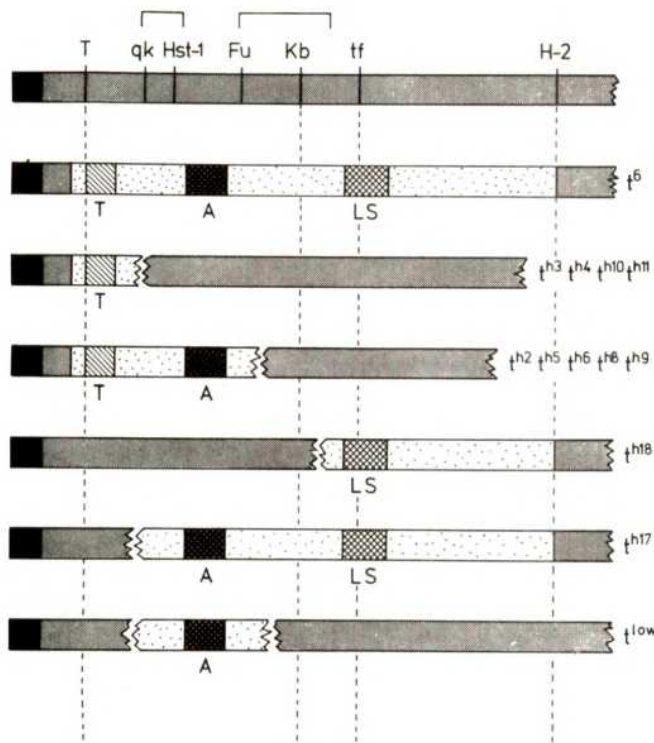
mutant haplotypes is derived from the haplotype t<sup>6</sup> (Fig. 1), which belongs to the t<sup>0</sup> complementation group<sup>9-13</sup>. Less detailed results with mutants from other haplotypes suggest that they may be broadly similar to t<sup>6</sup> (refs 14-16).

Lethal t-haplotypes are usually maintained in balanced lethal stocks, using crosses of Ttf/t<sup>x</sup> × Ttf/t<sup>x</sup>, both T/T and t<sup>x</sup>/t<sup>x</sup> being lethal. In such stocks mutant t's are occasionally found which have lost the lethality and are viable when homozygous. In all cases this loss of lethality is accompanied by crossing over between T and tf. This suggested the existence of a lethal factor (the LS-factor), located near the locus of tf, and lost whenever the wild-type allele of this locus is lost (Fig. 1, for example, haplotypes t<sup>h2</sup> and t<sup>h3</sup>). This was confirmed by the finding in linkage tests of the complementary type of crossover (t<sup>h17</sup> and t<sup>h18</sup>, Fig. 1)<sup>9,11</sup>. Mutant t's of this kind are lethal either when homozygous or in compound with t<sup>6</sup>, but they do not produce taillessness when heterozygous with T; thus, T/t<sup>h17</sup> and T/t<sup>h18</sup> are short-tailed like T/+. This leads to the interpretation that the T-interaction effect is due to a factor located near the locus of brachyury. Further study of the two complementary types of mutants revealed that all the viable mutants were male fertile, and t<sup>h17</sup> and t<sup>h18</sup>, which retained the lethality, also retained the male sterility of t<sup>6</sup>. It thus seems that a factor for male sterility is close to that for lethality<sup>10</sup>. For simplicity, the two factors are shown as one in Fig. 1 (the LS-factor), but it is possible that they are separable.

The next factor to consider is segregation distortion in males. Mutant viable haplotypes are of two main kinds (Fig. 1): those with normal ratio (t<sup>h3</sup>) and those with a low transmission ratio from males (t<sup>h2</sup>). This suggests that a factor for low ratio might be located between T and tf. This concept was confirmed by the discovery of the mutant low<sup>17</sup>, later renamed t<sup>low</sup> (ref. 10), which arose by crossing over in a stock carrying t<sup>h17</sup>. It has the property of causing a low transmission (<50%) from males of the chromosome on which it lies, but it is viable when homozygous, and does not modify the effect of T. t<sup>low</sup> is thus regarded as a middle-piece of t-chromatin carrying the abnormal ratio factor, designated the A-factor, but not the T- or LS-factors. The finding (mentioned below) of further t<sup>low</sup>-type mutants in the present work confirms this interpretation. In males carrying t<sup>low</sup> and another t<sup>x</sup> with an abnormal transmission ratio, either high or low, the two haplotypes t<sup>low</sup> and t<sup>x</sup> are transmitted with equal frequencies. Thus, the first requirement for abnormal transmission is that the A-factor should be present and heterozygous. However, the A-factor alone gives a low ratio. The high ratio seen in naturally occurring haplotypes seems to depend on other parts of the t-chromatin. Thus, it has been suggested that the spaces between the T-, A- and LS-factors are occupied by abnormal t-chromatin of some kind, and that the high transmission ratio of natural t-haplotypes is due to the presence of the A-factor and interaction of this factor with all parts of the t-chromatin<sup>9,10</sup>.

A further property of natural haplotypes so far unexplained is suppression of recombination over the segment of chromosome 17 from T to H-2. Mutant haplotypes which arise by recombination between T and tf usually permit recombination in this region, although still with some suppression. Such weak suppression could be due to partial loss of the crossover-

suppressing property over the whole T-H-2 segment, or mutant haplotypes might still suppress crossing over strongly over their own (reduced) length and allow free recombination elsewhere. If the latter explanation were correct, double heterozygotes for two complementary and overlapping mutant haplotypes (for



**Fig. 1** Diagrammatic representation of the structure of the  $t^6$ -haplotype, of various mutant haplotypes derived from it, and of the corresponding normal segment of mouse chromosome 17. The centromere is depicted to the left in black. Some relevant known loci in the normal chromosome are brachyury, T; quaking, qk; hybrid sterility, Hst-1; fused, Fu; knobby, Kb; tufted, tf; and H-2. In the t-chromatin the postulated T-int-, A- and LS-factors are hatched and the intervening stretches of altered chromatin are stippled.

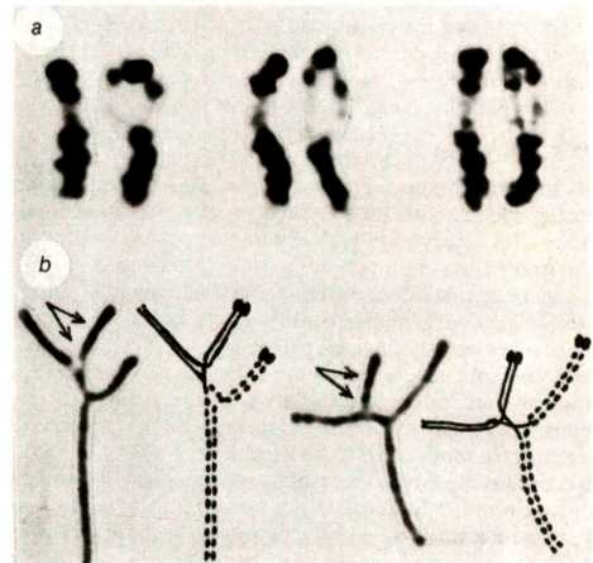
example,  $t^{h2}$  and  $t^{h17}$ , Fig. 1) should exhibit strong crossover suppression, even though each haplotype separately permits recombination. Conversely, in double heterozygotes for non-overlapping haplotypes (for example,  $t^{h2}$  and  $t^{h18}$ , Fig. 1), crossing over should occur freely in the segment of normal chromatin between the two stretches of t-chromatin. We have already presented evidence that in the double heterozygotes for  $t^{h18}$  and various viable T-end mutants, such as  $t^{h2}$ ,  $t^{h6}$  and  $t^{h11}$ , considerable crossing over in the relevant segment can occur<sup>9</sup>.

To test the overlapping haplotypes, animals heterozygous for  $t^{h2}$  and  $t^{h17}$  were bred. These particular haplotypes were known to overlap as both included the A-factor. The recombination between T and tf in these doubly heterozygous animals was then compared with the value in animals carrying  $t^{h17}$  only, and therefore having normal chromatin over part of the relevant distance (Table 1). In animals carrying only  $t^{h17}$ , the T-tf recombination is considerably enhanced (the normal value is about 8%), apparently an effect of the translocation T(1; 17)190Ca with which  $t^{h17}$  is inextricably associated. In the double heterozygotes  $t^{h2}/t^{h17}$ , however, recombination was strongly suppressed and was less than one-tenth of that in single  $t^{h17}$  heterozygotes ( $1.5 \pm 0.6$  compared with  $18.1 \pm 0.9\%$ , Table 1). The interpretation was that both  $t^{h2}$  and  $t^{h17}$  suppressed crossing over strongly over their own length, and thus, as the two haplotypes overlapped, recombination was strongly suppressed over the whole distance from T to tf.

## Other evidence

Hammerberg and Klein<sup>18</sup> showed that the crossover-suppressing effects of t-haplotypes extended beyond tf, approximately to the H-2 complex but little, if at all, beyond this. Thus, it is assumed that t-chromatin extends to H-2, but the absence of mutant haplotypes with breaks between tf and H-2 prevents a similar study of the basis of the crossover suppression in this segment. However, both  $t^{h17}$  and  $t^{h18}$  retain the H-2 haplotype of  $t^6$ , from which they were derived, even though they had been crossed to other stocks carrying other H-2 genes for many generations before their H-2 genotype was tested<sup>12</sup>. This is consistent with the idea that each t-haplotype suppresses crossing over for its own length, and that  $t^{h17}$  and  $t^{h18}$  extend distally as far as H-2, but it is not critical evidence.

There is little evidence concerning crossover suppression by mutants derived from natural haplotypes other than  $t^6$ . Bennett<sup>19</sup> bred animals doubly heterozygous for  $t^{38}$ , a viable mutant with low transmission ratio derived from  $t^0$  (ref. 15), and  $t^{low}$ . As  $t^{38}$  has a low ratio it must have included the A-factor (thus resembling  $t^{h2}$  in Fig. 1) and must have overlapped  $t^{low}$ . Bennett found no recombination between  $t^{38}$  and  $t^{low}$ , suggesting that  $t^{38}$ , like the  $t^6$ -mutants, suppresses crossing over throughout



**Fig. 2** a, Giemsa-stained chromosome 17 bivalents at pachytene. Examples of the synchronous and asynchronous synapsis observed in the large, pale 17B band in, left to right, +/+, T/+ and +/t<sup>6</sup> animals. Note also that in +/t<sup>6</sup> there is no detectable heterozygosity in the bivalent in length or staining intensity. b, Synaptonemal complexes and the diagrammatic interpretation of quadrivalents they form in T/+ and +/t<sup>w5</sup> T138 heterozygotes. Chromosome 17 and its translocated segments are shown as the continuous line, chromosome 9 and its segments as the broken line. Both quadrivalents show equivalent synapsis in the region of the 17B band (arrowed). The synaptonemal complexes were freed and fixed using the method described by Tres<sup>24</sup> but were then sedimented on to glass slides and stained with aqueous silver nitrate before viewing with the light microscope.

its own length. Pizarro and Dunn<sup>20</sup> showed that  $t^{w35}$ , a viable mutant derived from  $t^{w32}$ , decreased recombination in the T-tf interval and increased it in the tf-H-2 interval. This is consistent with the suggestion that  $t^{w35}$  suppressed crossing over strongly over its own length, leading to a compensatory increase in recombination in the adjacent segment.



**Table 1** The effect of overlapping t-haplotypes on T-tf recombination

Parents	Sex of heterozygote	Offspring				Total	Recombination (% $\pm$ s.e.)
		T+	Ttf	++	+tf		
T(t <sup>h17</sup> )/+tf $\times$ +tf/+tf	♀	399	79	84	352	914	17.8 $\pm$ 1.3
	♂	327	86	72	377	862	18.3 $\pm$ 1.3
T(t <sup>h17</sup> )/t <sup>h2</sup> tf $\times$ +tf/+tf	♀	70	—	2	81	153	1.3 $\pm$ 0.9
	♂	93	2	3	79	177	2.8 $\pm$ 1.2
		T++	T+tf	Tt <sup>h2</sup> +	Tt <sup>h2</sup> tf	Total	
+(t <sup>h17</sup> )/t <sup>h2</sup> tf $\times$ Ttf/+tf	♀	21	—	—	17	38	
	♂	44	—	—	41	85	
Combined data (t <sup>h17</sup> )/t <sup>h2</sup> tf	♀				RC	Total	
	♂				2	191	1.0 $\pm$ 0.7
					5	262	1.9 $\pm$ 0.8

Animals were classified for tail length within a few days (usually 1 day) of birth and for tufted, tf, at about 4 weeks. Any doubtful animals were kept and genetically tested. For the third group of crosses only the T-carrying offspring are shown as the remainder provide no linkage information. The seven recombinant animals in the crosses involving t<sup>h17</sup>/t<sup>h2</sup> were all genetically tested and shown to carry recombinant haplotypes. Both Ttf animals proved to be T(t<sup>low</sup>)tf/+tf. The 5++ animals all gave tailless offspring when crossed to T/+ (that is, they carried the T-int factor). Two permitted recombination between T and tf, and were shown to be due to chiasmata proximal to and distal to the overlap region; the remaining three are still being tested. RC, recombinant offspring.

Thus, the available evidence, though not conclusive, is entirely consistent with the suggestion that in any t-haplotype, whether natural or a derived mutant consisting of proximal, central or distal region, crossover suppression is co-extensive with the t-chromatin.

## Chiasma formation

In principle, crossover-suppression observed among the progeny may be due either to failure of chiasma formation or to normal chiasma formation with elimination of the products of crossing over.

Because at meiosis the chromosome 17 bivalent cannot be unequivocally identified, this question has been studied in t-haplotypes by marking the chromosome with translocations. In mouse translocation heterozygotes, if there is a chiasma in each arm of the multivalent then a ring is observed at meiotic metaphase I. If one arm lacks a chiasma then a chain configuration is formed, and if two arms lack chiasmata there may be either a chain-of-three plus univalent, or two unequal bivalents. Forejt<sup>21</sup> studied the configurations formed at first meiotic division in males heterozygous for T(9;17)138Ca, T(1;17)190Ca and T(8;17)43H. In each case animals carrying t<sup>12</sup> had a lower mean number of chiasmata in the translocated chromosomes than did their T/+ siblings. The reductions were 0.27, 0.25 and 0.45 chiasmata per multivalent in T138, T190 and T43, corresponding to map distances of 14, 13 and 23, respectively. Thus, they were approximately of the right order, as the recombination in T138 heterozygotes is reduced by about 9% when the haplotype t<sup>6</sup> is present<sup>22</sup>.

Because of the importance of this point, we have repeated Forejt's work, using T138 and three other t-haplotypes, t<sup>6</sup>, t<sup>w5</sup>

and t<sup>w32</sup>. Meiotic configurations in males heterozygous for T138Ca and a t-haplotype were compared with those in littermates or other close relatives heterozygous for T138 and T/+ (Table 2). In each case the number of chiasmata per multivalent was significantly lower in males carrying t<sup>6</sup>, t<sup>w5</sup> or t<sup>w32</sup> than in their T/+ relatives. For t<sup>6</sup> and t<sup>w5</sup> the reductions were 0.23 and 0.21 chiasmata per multivalent, respectively, quite close to the value found by Forejt for t<sup>12</sup>. With t<sup>w32</sup> the difference was greater, about 0.39 chiasmata, but as only two t<sup>w32</sup> males were studied, little importance can be attached to this point.

Our main conclusion is that, as a reduction in number of chiasmata in T138 multivalents is produced by four different t-haplotypes, t<sup>6</sup>, t<sup>12</sup>, t<sup>w5</sup> and t<sup>w32</sup>, then the crossover suppression caused by t is due to reduced chiasma formation, rather than elimination of products of chiasmata.

In other organisms factors which lead to chiasma suppression have been classified as asynaptic or desynaptic, according to whether synapsis fails or whether synapsis occurs normally but chiasmata do not form<sup>23</sup>. It would be valuable to know whether t-haplotypes should be considered as asynaptic or desynaptic. We attempted to study chromosome pairing in chromosome 17 of males with or without t by light microscopy of pachytene preparations. These studies revealed that the large pale Giemsa-staining band 17B pairs asynchronously even in normal (+/+ or T/+) animals and may be found unpaired when more proximal and distal bands are closely apposed (Fig. 2a). This is relevant as t-chromatin is thought to be located in this band (see below). However, there was no difference in pairing between +/t<sup>6</sup> or +/t<sup>w5</sup> and T/+ animals, either in these Giemsa-stained chromosomes or in synaptonemal complexes observed by light microscopy, using T138 as a marker of chromosome 17 (Fig. 2b). Thus, chiasma suppression in t-haplotypes is probably of

**Table 2** Multivalent configurations and chiasmata at first meiotic division in male mice heterozygous for T138Ca and various t-haplotypes

Haplotype	No. of males	RIV	No. of cells with configuration			Total	No. of chiasmata	Difference
			ChIV	ChIII+I	20II			
t <sup>w5</sup>	3	734	221	3	397	1,355	3.25	
Ttf	5	1,353	571	8	320	2,252	3.46	0.21
t <sup>w32</sup>	2	453	161	3	414	1,031	3.04	
Ttf	4	1,244	291	9	400	1,944	3.43	0.39
t <sup>6</sup>	7	2,176	570	22	1,189	3,957	3.24	
Ttf	5	1,860	452	16	503	2,831	3.47	0.23

Males were bred by crossing Ttf/t+  $\times$  T138/T138. Testis preparations were made by the method of Evans *et al.*<sup>34</sup> and stained with toluidine blue. Observations were made of cells in diakinesis-metaphase I using coded slides. RIV, ring of 4; ChIV, chain of 4; ChIII+I, chain of 3+univalent; 20II, no multivalent configuration.



the desynaptic type, but further work, probably involving electron microscopy, is needed to confirm this point.

The chiasma suppression of *t*-haplotypes is typically studied in heterozygotes, having normal chromatin on the homologous chromosome. It would be valuable to know whether chiasmata are also suppressed when *t*-chromatin is present on both homologues. Does the abnormality involve mutual recognition of *t*-chromatin and normal chromatin, or is the low chiasma-forming property of *t*-chromatin autonomous?

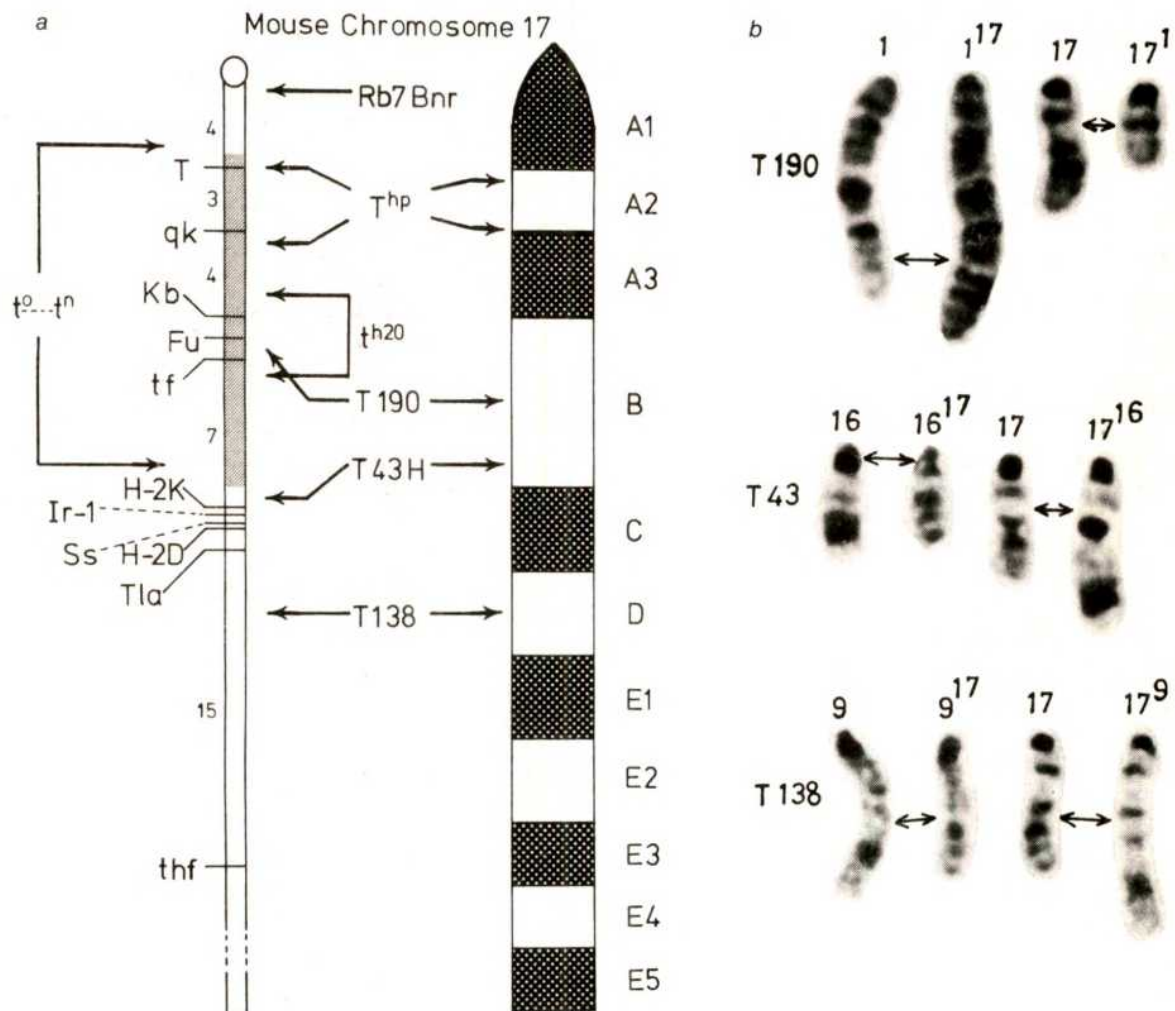
Crosses intended to test this point are in progress but have been inconclusive. Similarly, the crossover haplotypes derived from the  $t^{h2}/t^{h17}$  heterozygotes are being studied to determine whether any have resulted from chiasmata in the overlap region. To date, two  $t^{low}$  type mutants have been found (which give no information on chiasma position) and one each of mutants resulting from chiasmata proximal to and distal to the overlap region. It seems likely that chiasmata are suppressed at least to some extent, when *t*-chromatin is homozygous as well as when it is heterozygous, but further work is needed on this point.

### Location and extent of altered chromatin

Genetically, the crossover-suppressing effect of *t*-haplotypes extends from the *T* to *H*-2 loci, but apparently not far beyond

*H*-2 (ref. 18). There is little evidence about the effect proximal to the *T*-locus.

Cytogenetically, part (and perhaps all) of the *t*-chromatin lies in band 17B, the large band in chromosome 17, which stains lightly with Giemsa. The evidence for this comes from the relative location of translocation breaks and genetic markers (Fig. 3a). Translocations *T*(1; 17)190Ca and *T*(16; 17)43H have breaks in band 17B (refs 25, 26), and *T*(9; 17)138Ca in band 17D (ref. 25) (Fig. 3b). Genetically, the *T*138 break is 3cM distal to *H*-2 (ref. 27) and that in *T*43H is very close to *H*-2K (ref. 28). Thus, it seems probable that the *H*-2 complex, and hence the distal end of *t*-chromatin, lies near the distal end of band 17B. The locus of *tf* (near the centre of the *t*-chromatin) apparently lies distal to the *T*190 break, and hence also in 17B (ref. 29). The position of the brachyury locus is less certain. Bennett<sup>1</sup> reported that the brachyury allele, *T*<sup>hp</sup>, which involves a deletion extending from *T* to *qk*, was cytogenetically detectable as a shortening of the A2 band. However, we have been unable to confirm this. In preparations made from 12-day embryos, some *T*<sup>hp</sup>/*t*<sup>h2</sup> and others *+*/*t*<sup>h2</sup>, no consistent differences between the pairs of chromosome 17 were detected (Fig. 4). Hence, it is not clear whether the *T* locus lies in band 17A2, or whether it too lies in 17B. Thus, the stretch of altered chromatin in *t*-haplotypes probably occupies the whole of band 17B and may extend proximally into the 17A bands.

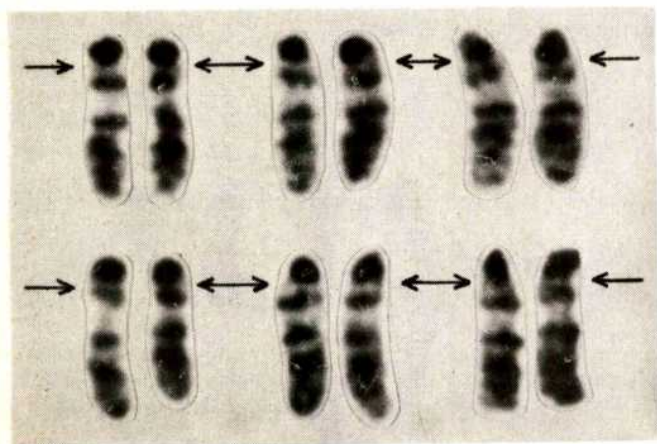


**Fig. 3** *a*, Genetic (left) and cytogenetic (right) maps of mouse chromosome 17. In the genetic map the segment thought to be occupied by *t*-haplotypes is shaded. The approximate positions of the breakpoints in *T*190Ca, *T*43H, and *T*138Ca are shown by arrows. The genetic positions were determined by pedigree analysis; the evidence for the cytogenetic positions is shown in *b*. Gene symbols as in Fig. 1 and also *Ir*-1, immune response-1; *Ss*, serum serological (factor C4 of complement); *Tla*, thymus leukaemia antigen; *thf*, thin fur. *b*, G-banded normal and translocated chromosomes showing breakpoints (arrowed) in *T*190Ca, *T*43H and *T*138Ca heterozygotes.



## Nature of the genetic changes

Although the exact mechanism remains unknown, it seems that t-chromatin alters the function of the chromosome at meiosis in such a way that chiasma formation is reduced. Moreover, as previously known, chromosome function in development is altered by t-chromatin, leading to embryonic lethality, modification of tail length and effects on spermatogenesis. In considering the type of change in the DNA which could produce such effects, it seems that major structural changes, such as inversions, are ruled out, as are long-range position effects acting from only one or two points. Such position effects could not explain the way in which effects on recombination change with length of haplotype, nor the fact that not only proximal and distal ends but also central fragments ( $t^{\text{low}}$ -mutants) can retain t-properties. It seems that some change repeated along the altered stretch of DNA in band 17B must be involved. DNA of higher organisms is thought to have five levels of organisation<sup>30</sup>: (1) unique sequences, including structural genes for proteins; (2) repeated genes coding for some types of RNA and protein for which multiple coding sequences are required; (3) moderately



**Fig. 4** Above, three G-banded homologous pairs of chromosome 17 from  $+/t^{\text{h}2}$  embryos and below, three from  $T^{\text{hp}}/t^{\text{h}2}$  embryos, showing no detectable heterozygosity in length or staining of the pale A2 band (arrowed).

repetitive sequences interspersed with the coding DNA (iDNA); (4) highly repetitive DNA, other than satellite DNA; (5) satellite DNA having highly repeated sequences and located in large blocks of chromatin.

We postulate that in t-chromatin it is the moderately repetitive DNA, that is, level (3) of organisation, in the chromosomal segment occupied by each haplotype, that is altered. The structural sequences in this segment seem to be present and normal. The evidence for this is that there are various known loci in the region occupied by t-haplotypes, such as the loci of qk, Kb and tf, for which t-chromatin seems to behave in a similar way to wild-type. Thus,  $t/qk$ ,  $t/Kb$ , and  $t/tf$  phenotypically resemble  $+/qk$ ,  $+/Kb$ , and  $+/tf$ , respectively. Therefore, one must assume that t-chromatin includes normal structural sequences for these loci. Conversely, a change in the iDNA could account for the observed phenomena. Such sequences are believed to be involved in the 'fine-tuning' of gene expression<sup>30</sup>, and in meiotic crossing over<sup>31</sup>. We postulate that t-chromatin differs from normal in such a way that chiasma formation is impaired, and in development the function of different structural loci is or is not materially altered, according to their dependence on the particular change in 'tuning', which might, for instance, involve an alteration in timing or rate of transcription. Many of the effects might be quantitative, but if a processing gene<sup>32</sup> was affected

then polypeptide structure might be altered. The lethality would result from one or more profoundly affected structural loci, the T-int effect from a slight effect on the T locus, whereas loci such as qk, Kb and tf, are not detectably affected.

Because the relevant sequences are repeated, any change in them need not necessarily be an alteration in the sequence, but might instead be an altered number of repeats, or a combination of these factors. Each naturally occurring t-haplotype, such as  $t^0$ ,  $t^{\text{w}1}$ , and  $t^{\text{w}5}$  must be considered as potentially having its own particular type of altered iDNA. Hence, the T-int-, A- and LS-factors would potentially exist in allelic forms, according to the haplotype from which they were derived.

The means by which naturally occurring t-haplotypes arise from wild type is problematic. For repetitive sequences some mechanism, not yet understood, may exist by which a change can occur throughout a chromosome segment, such as mouse chromosome 17B, in a single event. On the other hand, it is not certain that t-haplotypes do arise in a single event. In the laboratory, short stretches of t-chromatin found in mutant haplotypes are indefinitely stable. Therefore, it is possible that in the wild, short stretches arise and become elongated by unequal crossing over until they reach a length at which they have a high transmission ratio, by which they are then maintained in the wild population. The high transmission, combined with deleterious effects (lethality and sterility), gives the impression of a 'parasitic gene' (ref. 33), and this in turn raises the possibility that incorporated viral genomes may in some way be involved in t-haplotypes. However, any explanation along these lines would be purely speculative.

Finally, if t-haplotypes are viewed as alterations in iDNA they acquire a new significance in studies of mouse genetics. Such DNA forms a significant part of the genome and if, as postulated, it affects gene expression, it would be expected to have important effects on the phenotype. Little work has so far been done on this question: t-haplotypes may provide a suitable system for future work.

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# Expression in *Escherichia coli* of hepatitis B virus DNA sequences cloned in plasmid pBR322

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*Fragments of hepatitis B virus DNA isolated from Dane particles have been inserted into the Escherichia coli plasmid pBR322 and cloned. Cells carrying the hybrid plasmid synthesise antigenic material that reacts specifically with antisera to hepatitis B viral antigens.*

INFECTION with hepatitis B virus (HBV) is widespread in man. Between 3 and 15% of healthy blood donors in Western Europe and the US show serological evidence of past infection and about 0.1% are chronic carriers of the virus. In many African and Asian countries the prevalence is much higher and the majority of the adult population have been infected, while 5–10% of the population are chronically infected<sup>1</sup>. Most infections are subclinical and are followed by apparently complete recovery with the development of virus-specific antibody. However, a significant proportion of infections (probably 1–5%) may produce chronic sequelae including persistent infection, chronic hepatitis of various types, cirrhosis and possibly primary liver cancer.

Plasma from some blood donors and patients infected with HBV contains 42-nm spherical particles (Dane particles)<sup>2</sup> which have serological and biochemical properties, suggesting that they are the infective virions of hepatitis B. These have an outer envelope containing the hepatitis B surface antigen (HBsAg) and an inner core (diameter 27 nm) bearing a second unrelated antigen, the hepatitis B core antigen (HBcAg). Within the core is a double-stranded circular DNA molecule of molecular weight  $\sim 2 \times 10^6$  which has a large variable gap in one strand, and an endogenous DNA-dependent DNA polymerase activity that can fill in this gap in *in vitro* reactions<sup>3</sup>. There is some evidence that the total virus genome length may be around one-third greater than the  $2 \times 10^6$  daltons found in single molecules in which case productive infection of a cell may require simultaneous infection by at least two genetically different particles<sup>3</sup>. A third antigen, the hepatitis B e antigen (HBeAg), which is probably also virus-coded, is found free in the plasma of some infected individuals and possibly also in association with Dane particles. Passively or actively acquired antibody to HBsAg (anti-HBs) confers some immunity to subsequent HBV challenge, and prototype vaccines composed of purified inactivated HBsAg prepared from the plasma of infected carriers are being evaluated<sup>4</sup>. However, the virus cannot be grown in tissue culture and normally infects only man and apes. This means that

molecular studies of the virus and its genome have been based on the limited amounts of material obtainable from the plasma of infected individuals. Such studies could be advanced considerably by insertion of HBV DNA into a bacterial plasmid or phage to allow its production in quantity from cloned, purified single molecules. Such clones would also be useful for studies of the expression of HBV gene products in bacterial cells, and for large-scale production of HBV DNA and viral antigens for diagnostic purposes and, possibly, vaccine production.

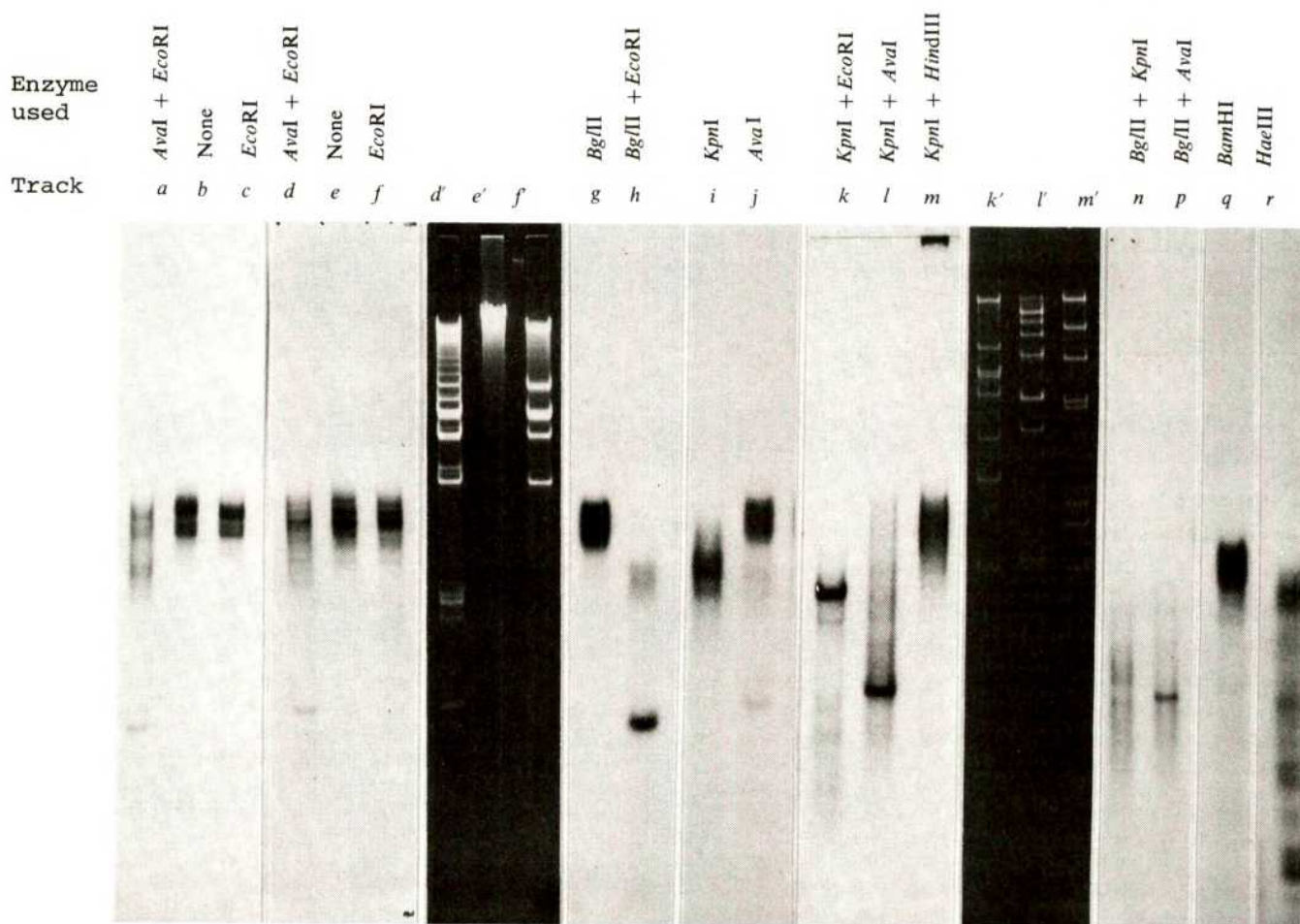
## Restriction of HBV DNA

The published information on digestion products of HBV DNA (Dane particle DNA) with restriction endonucleases<sup>5,6</sup> is limited and so additional analyses of this type were carried out before attempting cloning experiments. The amount of HBV DNA available was limited (about 90 ng from 5 ml of plasma), so the DNA was first labelled with <sup>32</sup>P in the endogenous DNA polymerase reaction<sup>3</sup>. DNA from bacteriophage  $\lambda$  was then added as a carrier to titrate the restriction enzyme and to provide reference fragments of known size. The digests were analysed by electrophoresis in agarose gels and the results of some of these experiments are shown in Fig. 1.

The heterogeneity of the undigested labelled HBV DNA (tracks *b* and *e*) precluded a detailed analysis of the restriction digests. The multiple bands of rather similar size could be due in part to the presence of linear and circular forms of otherwise similar molecules, in part to differing degrees of repair synthesis, although repeated DNA preparations from the same plasma sample gave reproducible patterns; the heterogeneity could also represent a true molecular dispersity from a mixed population of virions. HBV DNA preparations from several individual donors were all heterogeneous and differed slightly from each other (an example is included in Fig. 1; tracks *a*, *b* and *c* compared with *d*, *e* and *f*) both before and after digestion with various restriction enzymes. Heterogeneity has also been observed by Landers *et al.*<sup>6</sup> in the products of the endogenous polymerase reaction which comprised two principal radioactive components representing linear and circular molecules with additional minor components due to incomplete repair. Examination of the HBV DNA (Fig. 1, tracks *b* and *e*) by electron microscopy showed that the population contained circular molecules of MW  $\sim 2 \times 10^6$  and linear molecules ranging from 0.5 to  $10 \times 10^6$ . The linear molecules represented about three times the concentration of circular molecules and only a few of them had a MW around  $2 \times 10^6$ , the majority being between 0.5 and  $1 \times 10^6$ . The heterogeneity observed on electrophoresis was, therefore, not due to circular and linear forms of similar length and few, if any, of the linear molecules were labelled in the polymerase reactions.

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**Fig. 1** Autoradiographs of restriction enzyme digests of HBV DNA after electrophoresis in agarose gels. Examples of HBV DNA isolated from the plasma of two blood donors are shown, the samples in tracks *a*, *b* and *c* from one donor being directly comparable with those in tracks *d*, *e* and *f* from the other; the sample used for the digests in tracks *d*, *e* and *f* was also used for all the other digests shown, and also for the preparative experiments for cloning. In addition to the autoradiographs, the figure includes examples, indicated by primed letters, of the gels stained with ethidium bromide to reveal fragments of the  $\lambda^+$  DNA included with the HBV DNA in the restriction reactions. HBV DNA was prepared from Dane particles isolated by two cycles of ultracentrifugation from clarified plasma obtained from individual HBsAg-positive blood donors essentially as described by Landers *et al.*<sup>6</sup>. The single-strand gaps in the DNA molecules were repaired and the DNA radioactively labelled in reactions with the endogenous DNA polymerase in which  $^3\text{H}$ -dCTP and  $^3\text{H}$ -dTTP (22 and 30 mCi  $\mu\text{mol}^{-1}$ , respectively; Radiochemical Centre Amersham) or  $^{32}\text{P}$ -dGTP (300 mCi  $\mu\text{mol}^{-1}$ ) were included<sup>6</sup>. The released core particles were further purified by sedimentation through 30% w/v sucrose solution and DNA was isolated by treatment with proteinase K (Boehringer-Mannheim; 2 mg  $\text{ml}^{-1}$  in 0.6% SDS) followed by phenol extraction and dialysis. Restriction enzyme digests (37 °C, 1.5 h) were carried out in 10 mM Tris-HCl, pH 7.5, 10 mM  $\text{MgCl}_2$ , 10 mM 2-mercaptoethanol, 40 mM NaCl after addition of phage  $\lambda$  DNA (0.5–1  $\mu\text{g}$  per reaction) and the reactions were stopped by heating at 70 °C for 5 min. The samples were then applied to 1% w/v agarose gels<sup>23</sup> in 0.04 M Tris-acetate, pH 8.2 for electrophoresis (35 mA, 8 h). Gels were stained with ethidium bromide and photographed under UV light<sup>24</sup> and then either placed in alkali to denature DNA fragments for transfer to cellulose nitrate membrane filters<sup>13</sup> or dried on Whatman 3MM paper for autoradiography. The MW of the fragments of HBV DNA was estimated from their electrophoretic mobility<sup>24</sup> with reference to fragments of  $\lambda^+$  DNA in digests with *R.EcoRI* and *R.HindIII* included in the same gel<sup>25</sup>. These results are included in Fig. 2 which gives a provisional map of some of the restriction targets relative to each other.

Digestion of HBV DNA with *R.EcoRI* or *R.BglII* changed the pattern of the major bands only slightly (Fig. 1 tracks *c*, *f* and *g*) whereas digestion of the DNA with these two enzymes together (track *h*) gave a radioactive fragment, MW  $0.75 \times 10^6$  as the principal component, with little of the original DNA remaining. This is consistent with the introduction of a single break in circular molecules by either enzyme alone to give intact linear molecules; when the two enzymes acted together two fragments were formed, the smaller containing the entire labelled region. On digestion with *R.KpnI* (track *i*) almost the whole group of bands was displaced down the gel corresponding with the loss from each of a fragment of MW  $\sim 0.4 \times 10^6$  but no corresponding radioactive fragment was found. This suggests that HBV DNA may contain at least two targets for *R.KpnI* located outside the region repaired in the reaction with DNA polymerase and spanning a sequence common to all the molecules. The alternative explanation requiring cleavage of circular molecules at a single target to give linear molecules is less likely in view of the behaviour observed in the *R.EcoRI* and *R.BglII* digests. *R.HaeIII* furnished a spectrum of fragments with a range of sizes (track *r*) in a pattern broadly similar to that published<sup>5,6</sup>. Digestion of HBV DNA with *R.AvaI* and with

*R.BamHI* gave several radioactive fragments (tracks *j* and *g*); a major product of the *R.AvaI* digest had a MW of  $0.88 \times 10^6$ , while radioactive fragments of  $1.2 \times 10^6$  and  $1.8 \times 10^6$  occurred in the *R.BamHI* digests; in other digests with *R.BamHI* smaller fragments were also observed. These results, together with the principal radioactive products found in various digests with pairs of restriction enzymes (for example, *R.EcoRI* and *R.AvaI*, track *a* or *d*) are summarised in Fig. 2. Within the constraints imposed by the dispersity of the HBV DNA preparations used, they provided the approximation of relative positions of restriction targets as shown.

The results imply that, in most molecules, the single-stranded gap repaired by the endogenous DNA polymerase lies within a relatively constant region of the DNA sequence; Landers *et al.*<sup>6</sup> similarly concluded, from an analysis of *R.HaeIII* digests after different polymerase reaction times, that DNA repair took place largely within the same one-third to one-half of the total DNA sequence, although initiation of DNA repair could occur at variable sites within this region.

Digests of HBV DNA with *R.EcoRI* or *R.BglII* offer the possibility of cloning the entire HBV genome, while major fragments might be cloned from digests with *R.BamHI*, *R.KpnI*



or *R.AvaI*, or from various double digests. For structural studies of the HBV genome it is desirable to clone the entire DNA molecule, but clones covering a range of fragments could well be more useful for attempts to demonstrate the expression of HBV sequences in *E. coli*. Micromethods based on radioimmunoassay can be applied to individual bacterial colonies or phage plaques for the detection of specific polypeptides<sup>7</sup>. As many eukaryotic genes will not be expressed in prokaryotic cells it appeared desirable to insert HBV DNA fragments within a prokaryotic gene so as to produce a fused polypeptide; for this purpose the *R.Pst* target in the *E. coli* plasmid pBR322 has been used successfully<sup>8</sup>.

### Cloning of HBV DNA fragments in pBR322

HBV DNA isolated from Dane particles from a single HBsAg positive, HBeAg positive donor (serotype *adyw*) was labelled to a low specific radioactivity with <sup>3</sup>H in a repair reaction with the endogenous polymerase in order to facilitate its handling. This preparation was then variously digested with *R.EcoRI*, *R.BamHI*, *R.BglII*, *R.KpnI* and *R.AvaI*. Portions of the *R.EcoRI* and *R.BamHI* digests were used for insertion at the respective sites of appropriately restricted pBR322 DNA by annealing and ligation with T4 DNA ligase. The fragments from the other restriction enzyme digests and the remainder of the *R.EcoRI* and *R.Bam* digests were treated with polynucleotide terminal transferase for addition of 3' oligo (dC) sequences<sup>9</sup>. These fragments were annealed to pBR322 DNA to which oligo (dG) sequences had been attached after cleavage with *R.Pst*. The DNA preparations were then used to transform competent cultures of *E. coli* HB101 and transformants were screened for the acquisition of HBV sequences on the following basis. Cells transformed with recombinants made using the *R.Pst* site in pBR322 retained their resistance to tetracycline, but became sensitive to ampicillin. Cells transformed with recombinants made using the *R.BamHI* site in pBR322 became sensitive to tetracycline, but retained their resistance to ampicillin, while cells transformed with DNA cloned using the *R.EcoRI* site remained resistant to both antibiotics<sup>10</sup> and were detected by colony hybridisation<sup>11</sup> with <sup>32</sup>P-labelled DNA from Dane particles. The presence of HBV DNA sequences in colonies where antibiotic resistance and sensitivities indicated the insertion of additional DNA into the plasmid was confirmed by colony hybridisation.

### Characterisation of pBR322-HBV hybrids

Plasmid DNA isolated from cell cultures that had been treated with chloramphenicol to amplify plasmid production<sup>12</sup> was analysed by gel electrophoresis before and after digestion with restriction endonucleases.

DNA fragments from several of the gels were transferred to cellulose nitrate filters for hybridisation<sup>13</sup> with HBV DNA labelled with <sup>32</sup>P by the endogenous polymerase reaction. Examples of these results are shown in Fig. 3 and some of the characteristics of the cloned segments are given in Table 1. The hybridisation observed with appropriate fragments does not establish conclusively that the cloned sequences were HBV-specific, for the cloned DNA and <sup>32</sup>P-labelled probe had been prepared from the same plasma sample. Thus contaminating non-viral DNA fragments that had been cloned inadvertently would also have been detected if the same non-viral DNA sequence was labelled by the endogenous polymerase; however, in other experiments such <sup>32</sup>P-labelled probes hybridised with DNA from HBV-infected liver tissue, but not with DNA from normal human liver. Furthermore, preparations of <sup>32</sup>P-labelled DNA made from Dane particles purified from four different blood donors gave the same patterns when hybridised against restricted DNA fragments from the recombinant plasmids, thus strengthening the view that the cloned sequences were in fact HBV DNA.

The results presented in Fig. 3 are examples taken from the large number of recombinant colonies obtained from the transformation experiments and relate to colonies described below.

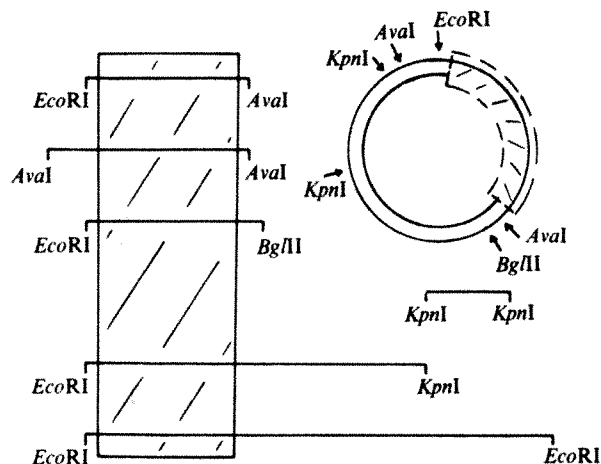


Fig. 2 The size and approximate relative order in the HBV genome of some of the major fragments in various restriction enzyme digests (Fig. 1) of HBV DNA. The site for *R.EcoRI* is taken arbitrarily as a reference point for the circular map. The shaded area indicates the region labelled with <sup>32</sup>P in the endogenous repair reaction.

### Expression of HBV sequences

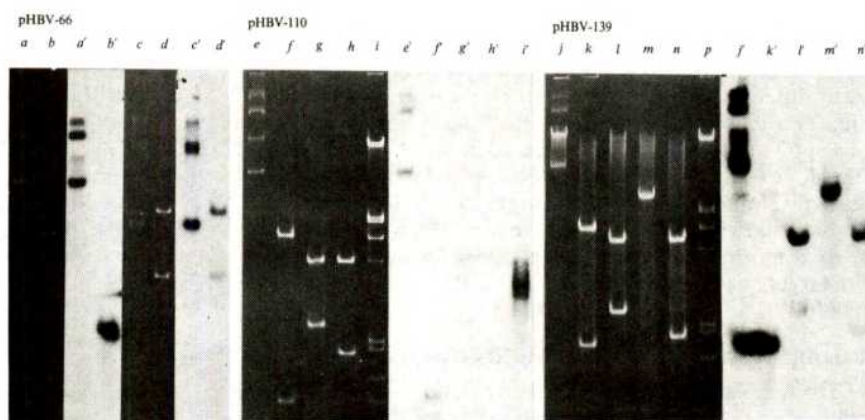
A population of the colonies carrying putative recombinant plasmids, selected on the basis of drug resistance and sensitivity characteristics, was screened for the production of HBV antigens. Three test systems were used in the solid phase method of radioimmunoassay described by Broome and Gilbert<sup>7</sup> which uses polyvinyl disks coated with IgG from specific antisera. These disks are placed in contact with cells producing antigen which binds to the IgG surface. When subsequently incubated with <sup>125</sup>I-labelled IgG from the same antiserum, the bound antigen retains the label and can be detected readily by autoradiography. The sera used were anti-HBs (human and hyperimmune animal sera), anti-HBc (human sera containing HBsAg and HBeAg and selected on the basis of a high anti-HBc titre) and anti-HBe and anti-HBe together (human sera containing HBsAg and high levels of anti-HBc and anti-HBe).

Clear positive results were obtained in the two test systems containing anti-HBc. Of some 350 colonies tested, 13 gave intense spots on autoradiography with the anti-HBc + anti-HBe antibodies (Fig. 4a). Most of these colonies remained strongly positive when re-tested in both the anti-HBc and anti-HBc + anti-HBe assay systems (Fig. 4b). None of the clones giving a negative response in the initial screening was subsequently positive, but some clones that were positive initially gave a negative result after subculturing, which probably reflects instability of some of the hybrid plasmids. All of the clones that were positive in the anti-HBc + anti-HBe system were positive when tested with anti-HBc alone, implying that none was producing detectable levels of HBeAg. If lysis of the bacterial colonies with phage  $\lambda$  before radioimmunoassay was omitted, only a very faint outline of the positive colonies was discernible (Fig. 4c). This is consistent with the presence of the antigen as a periplasmic polypeptide fused to the major part of  $\beta$ -lactamase (penicillinase) as anticipated. The sensitivity of this assay was shown to be comparable with that attainable with the same reagents in a solid phase microtitre well assay<sup>14</sup> by titration of 10  $\mu$ l samples (diluted progressively from 1 in 400) of semi-purified HBcAg from human liver<sup>15</sup>.

The immunological specificity of the reactions was confirmed by the following observations. Replicate assays with different anti-HBc sera identified the same positively reacting clones. The positive reactions in the HBcAg assay were abolished if the polyvinyl disks were coated with normal human IgG instead of specific anti-HBc IgG, or if the normal human serum used as diluent for the <sup>125</sup>I-labelled antibody was replaced by anti-HBc-positive serum from a different donor (by competition with excess unlabelled anti-HBc), or if <sup>125</sup>I-anti-HBs replaced <sup>125</sup>I-anti-HBc in the assay with the anti-HBc coated disks. Finally,



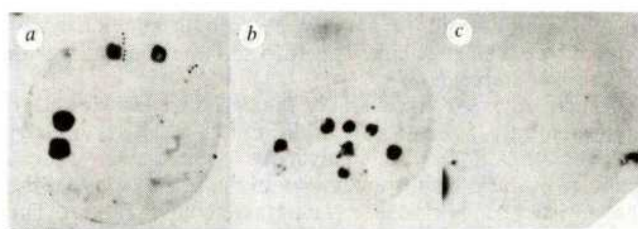
**Fig. 3** Electrophoretic separation in agarose gels of restriction enzyme digests of recombinant plasmids comprising pBR322 and HBV DNA sequences. The examples shown are of plasmids (Table 1) that elicit production of antigenic material that reacts specifically with antibodies to HBcAg (Fig. 4). The left-hand panels show DNA fragments revealed by staining with ethidium bromide and photography under UV light. After photography, gels were soaked in alkali for denaturation of the DNA fragments which were then transferred to cellulose nitrate membrane filters<sup>13</sup> for hybridisation with <sup>32</sup>P-labelled HBV DNA and autoradiography; the right hand panels show the corresponding radioautographs. HBV DNA (~0.5 µg) labelled with <sup>3</sup>H was prepared from 40 ml clarified blood plasma from a donor with a high titre of HBsAg, serotype *adw* as described in the legend to Fig. 1 and ref. 6. In one experiment 0.5 µg *E. coli* DNA was added as a carrier, but in a second the carrier was omitted. The DNA was divided into six portions for various restriction enzyme digests. Portions of *R. EcoRI* and *R. BamHI* digests were incubated with pBR322 digested with the same enzyme in reactions with T4DNA ligase<sup>26</sup> (1 U ml<sup>-1</sup>, 10 °C for 3 h followed by storage at 0 °C) in 66 mM Tris-HCl pH 7.2, 10 mM MgCl<sub>2</sub>, 40 mM NaCl, 0.2 mM EDTA, 0.1 mM ATP, 10 mM 2-mercaptoethanol. The remainder of the *R. EcoRI* and *R. BamHI* digests as well as *R. KpnI* and *R. BglII* digests were used in terminal transferase reactions, but with the exception of the *R. KpnI* digest the samples were first incubated with phage λ exonuclease (1.5 h at 0 °C in 50 mM Na glycinate, pH 9.5, 5 mM MgCl<sub>2</sub>, 50 µg ml<sup>-1</sup> bovine serum albumin (BSA) followed by phenol extraction and recovery of the DNA by ethanol precipitation) to remove the 5' single-stranded projections left by the restriction enzymes. Poly(dC) sequences were attached to the 3' termini by incubation with polynucleotide terminal transferase<sup>9</sup> (250 U ml<sup>-1</sup> for 10–20 min at 27 °C) in 15 µl 100 mM potassium cacodylate, pH 7.0, 1 mM CoCl<sub>2</sub>, 1 mM dCTP, 50 µg ml<sup>-1</sup> BSA, and the reactions stopped by addition of excess EDTA. An approximately molar equivalent of pBR322 DNA digested with *R. Pst* and incubated in similar reactions with dGTP instead of dCTP (from Dr J. van den Berg) was then added and the samples diluted to 50 µl for annealing in 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 5 mM EDTA, heated to 60 °C and gradually cooled to room temperature over a period of about 5 h. Aliquots of the solutions (10 µl) were then incubated with competent cultures (0.1 µl) of *E. coli* HB101 prepared as described by Lederberg and Cohen<sup>27</sup> and incubated overnight at 37 °C on L-agar plates containing tetracycline (20 µg ml<sup>-1</sup>) or ampicillin (50 µg ml<sup>-1</sup>). Colonies were subcultured on to Millipore filters supported on appropriate agar plates to test their resistance or sensitivity to the two antibiotics and for colony hybridisation<sup>11</sup> with <sup>32</sup>P-labelled HBV DNA from Dane particles. Colonies giving positive hybridisation reactions were grown in liquid culture and then shaken overnight at 37 °C after addition of chloramphenicol (170 µg ml<sup>-1</sup>) to amplify the number of plasmids within the cells which were then collected by centrifugation and treated with lysozyme and EDTA<sup>12</sup>. The resulting spheroplasts were lysed with Triton X-100 and the plasmid recovered by equilibrium centrifugation in CsCl solution (0.95 g per ml lysate) containing ethidium bromide (200 µg ml<sup>-1</sup>). The plasmid bands were collected, extracted with propan-1-ol saturated with aqueous CsCl, dialysed, and samples digested with restriction endonucleases as described in the legend to Fig. 1. The results shown are from the following hybrid plasmids: pHBV-66 in tracks a, b, c and d in which a is the undigested plasmid and b, c and d are digests with *R. Pst*, *R. KpnI* and *R. BamHI*, respectively; pHBV-110 in tracks e, f, g and h, in which e is the undigested plasmid and f, g and h are digests with *R. Pst*, *R. BamHI* and *R. BamHI* + *R. EcoRI*, respectively; pHBV-139 in tracks j, k, l, m and n, in which j is the undigested plasmid and k, l, m and n are digests with *R. Pst*, *R. BamI*, *R. EcoRI*, and *R. BamHI* + *R. EcoRI*, respectively. Tracks i and p show reference digests of λ<sup>+</sup> DNA with *R. EcoRI* + *R. HindIII*<sup>25</sup> which also contained <sup>32</sup>P-labelled undigested DNA from Dane particles. The primed letters identify the samples on the autoradiograph, made on Kodak X-omat H X-ray film with an intensifying screen, of the cellulose nitrate filters after hybridisation with HBV DNA.



absorption of the radioactive anti-HBc with semipurified HBcAg<sup>15</sup>, by overnight incubation at 4 °C followed by centrifugation to remove the excess antigen, abolished the positive result in the radioimmunoassay. Thus the detection of an as yet unidentified HBV-specific antigen by interaction with its specific antibody present in the sera of HBV-infected individuals remains a formal possibility, but it is unlikely, and the results are wholly consistent with the observed activity being that of HBcAg. The HBcAg polypeptide detected in the colonies, however, is unlikely to be identical with that occurring naturally for the cloning experiment was such as to produce polypeptides linked to β-lactamase<sup>8</sup>. However, it has not been established that this is the case and it remains possible that translation of β-lactamase sequences could be terminated and followed by reinitiation, but against this is the occurrence of clones that do not exhibit HBcAg activity, but which contain HBV DNA fragments similar to those that do. The point will be clarified by DNA sequence determination, which is in progress. All of the 13 HBcAg-positive clones had been made using the *R. Pst* site in pBR322, five with fragments from *R. BamHI* digests of HBV DNA and eight from *R. KpnI* digests. Not all of the plasmids have been isolated for analysis, but the three illustrated in Fig. 3 were all from cells giving positive reactions for HBcAg. The smallest fragment of HBV DNA in these plasmids (Table 1) was about  $0.95 \times 10^6$  daltons, but others with an HBV fragment

about half this size also gave positive reactions for HBcAg; values reported<sup>3</sup> for the MW of the naturally occurring HBcAg range from 17,000 to 80,000.

In an equivalent assay for HBsAg, similar, but faint positive reactions were obtained with four clones which are being analysed further. One might expect detection of expression of serological activity to be more difficult with HBsAg than with HBcAg as its protein moiety is markedly hydrophobic and hence



**Fig. 4** Autoradiographs showing the detection by radioimmunoassay of bacterial colonies expressing HBcAg. a, Four HBcAg-positive colonies amongst 52 colonies examined on one plate. b, The result obtained when initially positively reacting colonies, together with a random selection of colonies giving a negative reaction, were subcultured from stock plates and re-tested. c, A duplicate of b in which lysis of the colonies with phage λ before radioimmunoassay was omitted. Colonies of the bacteria were grown at 37 °C overnight on Millipore filters supported on nutrient plates containing tetracycline (to maintain selection for the plasmid). The cells were lysed by imprinting the filters for a few minutes on a lawn of bacteria confluent lysed with a virulent derivative of phage λ and then incubating further for several hours at 37 °C. Polyvinyl disks coated with anti-HBc + anti-HBe specific human IgG (60 µg ml<sup>-1</sup>) were placed face down on the colonies, which had obviously lysed, and incubated at 4 °C for 3–4 h. The disks were then removed and washed thoroughly and vigorously to remove the considerable quantity of adherent viscous bacterial debris. Finally the disks were incubated overnight at 4 °C with homologous <sup>125</sup>I-labelled IgG (10<sup>5</sup> c.p.s. per µg,  $2 \times 10^4$  c.p.s. per ml), washed thoroughly and autoradiographed on Kodak Blue X-ray film exposed for 2 d with an intensifying screen.

**Table 1** Some properties of the plasmids shown in Fig. 3

Hybrid plasmid	MW of fragment excised by <i>R. Pst</i> × 10 <sup>-6</sup>	Targets for restriction enzymes within the HBV sequences		
		<i>R. EcoRI</i>	<i>R. BamHI</i>	<i>R. Aval</i>
pHBV-66	1.2	—	+	+
pHBV-110	0.95	—	+	+
pHBV-139	1.16	—	+	+

In all the plasmids, the site for *R. BamHI* within the HBV sequence is located about  $0.7 \times 10^6$  daltons from the *R. Pst* site near the *R. EcoRI* site.



tends to remain associated with lipid<sup>16</sup>, and some experiments (but not others) suggest that carbohydrate residues on HBsAg glycoprotein may be required for full serological activity<sup>17,18</sup>.

## Conclusions and further implications

A large number of hybrid DNA molecules comprising pBR322 and various fragments of the HBV genome have been cloned and propagated in *E. coli*. It thus becomes possible to produce HBV DNA in the quantities required for detailed structural and sequence analysis and location of the various coding sequences in the viral genome. Such analysis with DNA from several independent isolates and clones will explain the basis of the heterogeneity found in DNA from Dane particles. The DNA will also be useful for further genetic manipulation related to studies of expression and as a source of a highly radioactively labelled probe for hybridisation experiments for both diagnostic purposes and fundamental studies.

When inserted within a normal coding sequence of the *E. coli* plasmid the DNA from hepatitis B virus, which normally infects only man and apes, can be expressed to give serologically active translation products. This suggests that at least the region of the HBV genome coding for the amino acid sequence necessary for serological activity probably does not contain inserted sequences, or 'introns', which have now been found in a number of eukaryotic and viral genes<sup>19-22</sup>, for it is widely believed, although not proved, that *E. coli* cannot process transcripts of these sequences. None of the hybrid plasmids so far examined contains a target for *R. EcoRI* within the HBV sequences. It was thus possible to insert the entire plasmid into a derivative of bacteriophage  $\lambda$  using this restriction site and plaques of the recombinant phage gave positive reactions in the disk radioimmunoassay (results not shown). The HBV sequences may well prove more stable when propagated within the phage genome, especially as a lysogen, and appropriate exploitation of the phage regulatory systems should permit significantly increased yields of the antigens from *E. coli*, raising the possibility of large scale antigen production for diagnostic purposes and development of vaccines. The insertion of HBV DNA into a phage  $\lambda$  derivative has been described very recently by Fritsch *et al.*<sup>28</sup>.

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# letters

## A 3-s delay in an optical burst from X-ray burst source MXB1735-44

THE discovery of optical bursts from the X-ray burst source MXB1735-44 was reported in a previous article<sup>1</sup>. One burst event was detected in both X rays and optical light. We showed (in Fig. 1 of the earlier article) that the optical burst is delayed by  $\approx 1.5$  s relative to the X ray burst. We concluded that to within the accuracy of the SAS 3 quick-look data timing ( $\pm 1$  s), the onset of the optical burst was coincident with that in X rays. A few months after the completion of our earlier paper, SAS 3 production data became available. We here report a re-analysis of the arrival times of the X ray and optical bursts based on these data which contain the universal time to an accuracy of  $\leq 5$  ms. We now find (as shown in Fig. 1) that the optical burst is delayed significantly by 2.8 s.

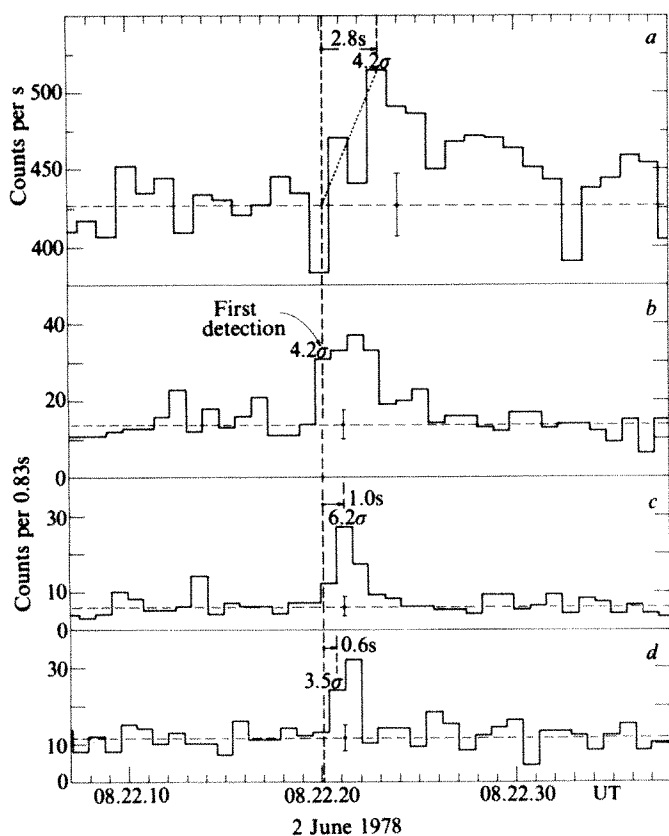
The optical observations were performed using the 1.5-m telescope at the Cerro Tololo Interamerican Observatory and a red-blocked RCA 31034 photomultiplier tube which had an

effective bandpass including the transmission of the atmosphere of 3,100-5,500 Å (FWHM). The absolute time, which was maintained to within 10 ms of UT by monitoring WWV, and the optical data in 100-ms integrations were recorded on a digital tape. Simultaneous X-ray observations were made with the horizontal tube detectors aboard the SAS 3 observatory<sup>2</sup>. The three energy channels of interest are 3-6 keV, 6-12 keV and 8-19 keV which have time resolutions of 0.42 s, 0.83 s and 0.83 s, respectively. The X-ray production data are assigned absolute times in a three-step process. First, real time X-ray data and timing data from the spacecraft clock are tagged with the absolute time at the NASA ground station in Quito, Ecuador, by recording them simultaneously with the output of a UT caesium clock. Second, in the production analysis at Goddard Space Flight Center the real time data are used to assign UT to the bulk of the data which are played back from the on-board tape recorder. Third, corrections for the variable VHF propagation time from the satellite to the ground station are made using a satellite ephemeris based on four days of radar-Doppler track-

ing data. Atmospheric propagation delays limit the accuracy of the absolute timing of the production data to  $\pm 5$  ms of UT.

In the burst event shown in Fig. 1, the optical burst was delayed by 2.8 s relative to the 3–6-keV X-ray burst and  $\approx 2.0$  s relative to the 6–19-keV burst. Defining the moment of detection as the time of the first bin in which there is a  $\geq 3\sigma$  increase above the mean background counting rate, the burst was detected first in the 3–6-keV band at 08.22.20.2 UT. The higher-energy X rays were delayed by  $\approx 1$  s and the optical pulse was delayed by  $2.8 \pm 0.5$  s. The  $\pm 0.5$ -s uncertainty ( $\sim 2\sigma$ ) in the optical delay was derived by examining alternative binning schemes for the optical and the X-ray data which have fundamental time resolutions of 0.1 s and 0.42 s, respectively. One sigma gaussian error bars are shown in Fig. 1. Also, the exact Poisson statistical significance of the first bin to exceed  $3\sigma$  in each bandpass is given. The dotted line in Fig. 1 shows that the optical data are equally consistent with a 3-s linear rise to maximum from the time the burst was first detected or with a 3-s delay followed by an abrupt rise to maximum. The optical burst has about twice the duration of the X-ray burst. About half of the total optical burst flux was detected at a statistical significance of  $4.0\sigma$  during the 7-s interval from 08.22.25.5 to 08.22.32.5 UT, well after the X-ray flux had ceased.

The higher energy X-ray pulses are delayed  $\sim 1$  s relative to the 3–6 keV pulse and decay  $\sim 1$  s sooner. The X-ray data are consistent with a black body emitter which is heated in  $\sim 1$  s, reaches a peak temperature  $\sim 30 \times 10^6$  K and subsequently cools



**Fig. 1** X-ray/optical burst from the X-ray burst source MXB1735–44. The event was detected first in 3–6-keV X rays, as marked by the vertical dashed line, and  $\approx 2.8$  s later in blue light. The background counting rates and their approximate ( $N^{1/2}$ ) uncertainties are indicated by horizontal dashed lines and error bars, respectively. The exact Poisson statistical significance of the first bin to exceed a  $3\sigma$  detection threshold is also given. The dotted line shows that the optical data are equally consistent with a 3-s linear rise to maximum from the time the burst was first detected or with a 3-s delay followed by an abrupt rise to maximum. A large tail is apparent in the optical data (see text). *a*, Optical 3,100–5,500 Å; *b*, 3–6 keV; *c*, 6–12 keV; *d*, 8–19 keV.

in  $\sim 1$  s. This agrees with detailed studies of the X-ray spectra of several burst sources which show that the burst emission near maximum is best fitted by a  $25\text{--}30 \times 10^6$  K black body<sup>3–5</sup>.

The optical pulse is probably produced by the reprocessing of the X-ray pulse into light in an accretion disk or in the photosphere of an optical companion. The  $\sim 3$ -s delay of the optical pulse may then be attributed to one or both of the following effects: (1) light travel time in the system and (2) the time required to reprocess X rays into light. (We neglect propagation delays due to the interstellar medium which are probably negligible as shown by simultaneous optical and X-ray observations of the Crab pulsar<sup>6</sup>.) For relatively neutral matter, in the outer portion of an accretion disk ( $R \geq 1$  light s) or in the photosphere of a companion star, the reprocessing time for the bulk of the photon flux would be small ( $< 1$  s). This is a consequence of the soft ( $\sim 30 \times 10^6$  K) black body spectrum of the burst radiation discussed above. Two-thirds of the photons are below 8 keV ( $\sim 40\%$  of the energy) and would be photoelectrically absorbed by C, N and O at small optical depths<sup>7</sup>. The third of the photons above 8 keV ( $\sim 60\%$  of the energy) would be reprocessed more slowly<sup>7</sup>, and might contribute to the observed optical tail discussed above.

If the optical pulse originates in the atmosphere of a stellar companion, then it will be delayed due to light-travel time by an amount

$$\Delta T \approx L/c[1 - \cos \phi \sin i]$$

where  $L$  is the average distance of the X-ray star from the photosphere of the companion and  $\phi$  and  $i$  are the orbital phase and inclination angles, respectively. We have assumed that the orbit is circular and that the stellar radius is not too large compared with the binary separation. For any  $i$  and over the range of  $\phi$  for which a significant portion of the heated photosphere is visible ( $120^\circ \leq \phi \leq 240^\circ$  for large  $i$  and the full range of  $\phi$  for small  $i$ )<sup>8</sup>,  $\Delta T = 3$  s gives:

$$1.5 \text{ light s} \leq L \leq 6 \text{ light s}$$

or

$$0.6 R_\odot \leq L \leq 2.6 R_\odot$$

The model predicts an observable modulation ( $\geq 25\%$  for  $i \geq 15^\circ$ ) of the delay time at the orbital period. As we noted in our earlier paper<sup>1</sup>, the lack of photometric variability ( $< 0.1$  mag) in the steady optical source<sup>9,10</sup> argues against this model and suggests that an accretion disk may contribute significantly to the optical luminosity and/or shield much or all of the stellar surface.

Alternatively, the optical pulse could be produced in the outer portion of an accretion disk ( $R \sim 2\text{--}3$  light s). In this case the time delay would show at most a small modulation at the orbital period (see DQ Her in refs 11, 12). It may also be variable, if for example, the burst emission is beamed or if there are changes in the structure of the disk.

The optical pulse might also originate in the fully ionised inner portion of an accretion disk ( $R \leq 1$  light s) which is not shielded from the compact source and in which the reprocessing time is long. The results of steady-state calculations for uniform density models scaled to high densities<sup>13,14</sup> and for models of X-ray illuminated atmospheres extrapolated to high fluxes<sup>15</sup> suggest that irradiated matter within  $\sim 1$  light s of the compact source will be thick to Thomson scattering and thin to photoelectric absorption. In these conditions the burst X rays which are not reflected from the disk will undergo many Thomson scatterings, and the bulk of the optical photons will be produced at large optical depths. Detailed calculations are required to estimate how these and other reprocessing effects would determine the delays, widths, rise times and intensities of optical bursts.

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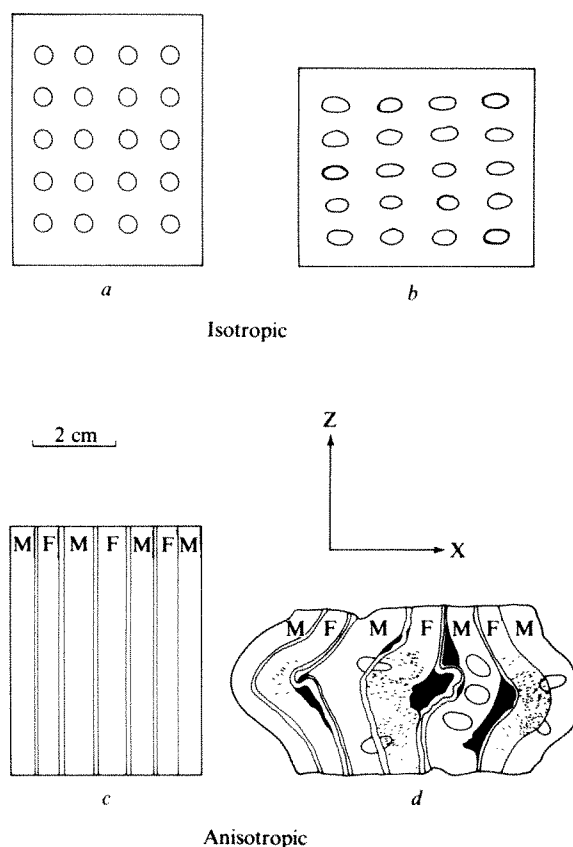
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## Experimental folding in ice and the resultant *c*-axis fabrics

FOLDS are commonly observed features of naturally deformed rocks<sup>1</sup> and glaciers<sup>2,3</sup>. Many different materials<sup>4</sup> have been used to model naturally occurring fold patterns. The deformation experiment described here used ice as an analogue for quartz in quartz-rich rocks by deforming a sample with an initially planar layering or anisotropy. This deformation is compared with samples of unlayered isotropic polycrystalline ice (Fig. 1a). Ice is convenient to use in the laboratory because of its relative ease of deformation at readily controlled temperatures. When used to model the behaviour of quartz-rich rocks, ice is a realistic analogue because both ice and quartz have: (1) hexagonal crystal structures; (2) exhibit similar optical properties; (3) form polycrystalline aggregates; and (4) deform using common crystallographic slip planes, particularly the (0001) basal planes<sup>5,6</sup>. In our experiments, a sample of multilayered ice consisting of plates of fine-grained ice sandwiched between layers of coarser ice (Fig. 1c) was deformed in a plane strain apparatus. The *c*-axis orientations of the grains were initially random and therefore the composite sample resembled the structure of many naturally occurring quartzites<sup>7</sup>. The experimental technique described here makes it possible to explore the relationship between initial anisotropy on folding and fabric development.

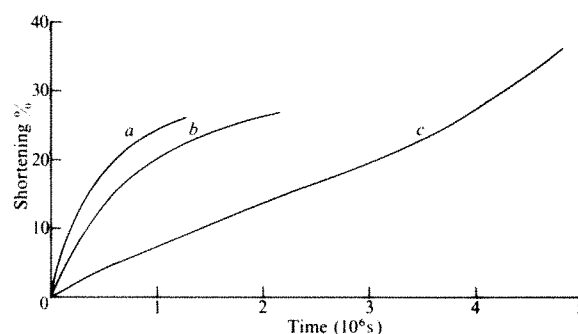
Cylinders of polycrystalline ice were prepared from a mixture of sieved ice-crushings and distilled water (details of the method will be published elsewhere). The ice contains equant grains of essentially uniform grain size with randomly orientated *c*-axes. The grain size can be controlled and for these experiments cylinders with grain sizes of 1 mm (fine grained) and 2 mm (medium grained) were produced. An irregular distribution of small air bubbles between 0.1 and 0.2 mm diameter were



**Fig. 1** a, An isotropic block of fine-grained ice with circular strain markers. b, The isotropic sample after 21% shortening and the strain markers are now ellipses. c, The planar fine- and medium-grained layers in the anisotropic block, separated by the films of frozen water. d, The anisotropic ice after 36% shortening. Voids have developed between layers (black), and air bubbles are more elongate (fine lines) in the hinge region of the fine-grained layers. Only a few elliptical strain markers have been preserved on this *XZ* face after deformation.

present at an estimated concentration of  $10 \text{ mm}^{-3}$ , in the grain boundary areas, in parts of the fine-grained ice.

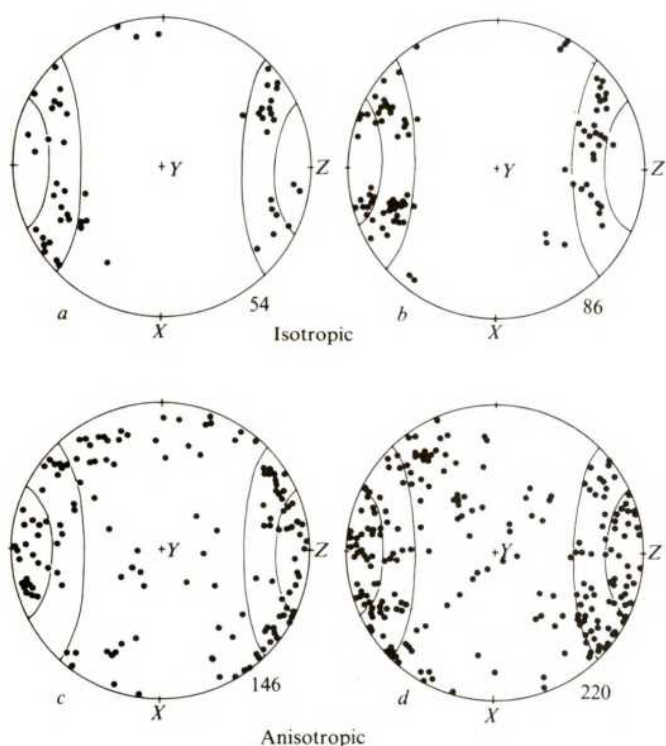
Each cylinder was cut into plates  $\sim 6 \text{ mm}$  thick and also a block  $\sim 50 \times 50 \times 60 \text{ mm}$ . A composite layered block of comparable dimensions was made from the two sets of plates. After being cut flat on a Leitz microtome, the surfaces of each plate were coated with a 1% solution of polyvinyl formate (Formvar) dissolved in ethylene dichloride and allowed to dry for 12 h. The surfaces were then frozen together with a film of distilled water to produce an anisotropic block of alternate fine- and medium-grained layers (Fig. 1c).



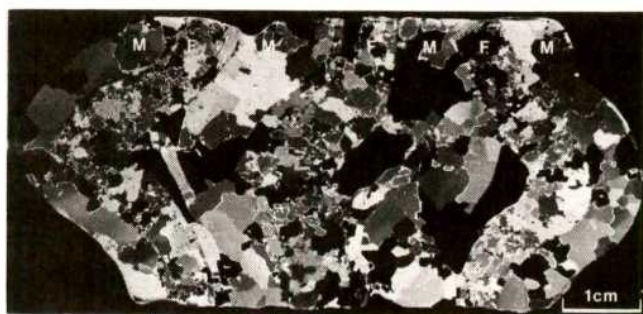
**Fig. 2** Displacement-time curves for blocks of fine-grained (a) and medium-grained (b) isotropic ice shortened by 27% and anisotropic ice (c) shortened by 36%.

Strain markers consisting of circular grooves 5 mm diameter, 0.4 mm deep and 0.3 mm wide, were inscribed at 10 mm centres on the *XZ* faces of the three blocks. (Here the extension direction is *X*, the direction of no length change *Y*, and *Z* is the shortening direction). The blocks were then deformed in a plane strain apparatus similar to those described by Kamb<sup>8</sup>, and Budd and Matsuda<sup>9</sup>. The layers in the composite block were parallel to the *YZ* plane. The load on the layered block was 83 kg and that on the isotropic samples 150 kg and so the initial stresses were 0.4 MPa and 0.6 MPa respectively. During loading and unloading the temperature was typically  $-10^{\circ}\text{C}$ , and for the deformation it was controlled to  $-1.00 \pm 0.05^{\circ}\text{C}$ . The displacement-time curves of the fine-grained and medium-grained isotropic ice are of a similar decelerating form, whereas the more lightly loaded anisotropic sample deformed at an increasing rate after 20% shortening (Fig. 2). This is presumably attributable to buckling and increasing instability of the layers.

The isotropic ice deformed homogeneously on a scale greater than a few grain diameters. The numerous small amplitude undulations (parallel to *Y*) that formed on the *YZ* faces of both the fine- and medium-grained samples are the only inhomogeneities observed, and have amplitudes and wavelengths of the order of the average grain size. The uniform distortion of the circular strain markers into a set of ellipses suggests that extension in the *X* direction was uniform. The bubbles in the fine-grained sample were elongated parallel to the strain ellipses. Grain sizes were uniform and boundaries were slightly irregular. Occasional deformation bands developed in individual grains as sharply delineated, wide lamellae. The *c*-axis orientation patterns of deformed fine- and medium-grained isotropic samples were similar (Fig. 3a and b), and were found not to vary throughout each sample. The majority of *c*-axes are distributed between the 25 and 45° cones centred about the shortening axis. This pattern is also consistent with other plane strain experiments with shortenings of greater than 40% (unpublished data).



**Fig. 3** *c*-axis patterns of preferred orientation. (a) and (b) show the fine- and medium-isotropic ice shortened by 27%. (c) and (d) show the fine and medium layers in the anisotropic ice shortened by 36%. The data are plotted on the lower hemisphere equal area projection and the superimposed small circles are at angles of 25° and 45° to the shortening axis *Z*. The number of *c*-axes is shown adjacent to the projection.



**Fig. 4** Microstructure in the folded fine- and medium-grained layers of the anisotropic ice. An interlocking grain aggregate in the fine layers is in marked contrast to the large grains of the medium layers.

The anisotropic specimen deformed inhomogeneously and large symmetric sinusoidal folds were produced in the medium- and fine-grained layers (Fig. 1d). The folds are disharmonic and their axial surface lies approximately perpendicular to the direction of shortening. Folds in the outer layers are parallel and penetrative, and their hinge lines make a 20° angle to *Y* (in the *XY* plane). Fold amplitude and sense of closure change in the *Y* direction in the central portion of the block, where dilation voids also appear between layers. The thin ice films used to bond the fine- and medium-grained layers together have also been folded, partly in conformity with adjacent fine- and medium-grained layers. However, adjacent to the voids the ice films display minor folding where axial surface orientations vary from perpendicular to parallel to the shortening direction.

The strain distribution within individual layers is inhomogeneous with the strain ellipses being distributed about the axial surface of the major folds. Where an original circle lay across the boundary between a fine- and medium-grained layer it was either significantly elongated in the fine layer or displaced parallel to the layer boundary. The small air bubbles in the fine-grained layers are elongate and describe a symmetric fan-like pattern.

In the anisotropic ice sample the relative grain size difference between adjacent layers has been preserved. Grain shapes are quite irregular and grain diameters have increased (Fig. 4). In the fine layers the average grain size is 1.5 mm, and in the medium layers grains vary from 2.5 to 4.5 mm. Nearly every grain contains undulose extinction or deformation bands. The *c*-axes in both the fine and medium layers (Fig. 3c and d) are distributed throughout the projection with a strong maxima parallel to the shortening direction. Such a pattern is a significant departure from the small circle distribution of the isotropic samples. An axial distribution analysis<sup>10</sup> of the anisotropic samples suggests that there is no relationship of *c*-axis orientation to relative position in the fold. The pattern of *c*-axes is consistent with some patterns recognised in natural<sup>7</sup> and experimentally deformed<sup>11</sup> polycrystalline aggregates of quartz and also natural ice<sup>12</sup>.

Folding induced by an initial layering produces a different fabric from a homogeneously deformed polycrystalline aggregate. This effect on fabric development has not been considered in other experimental studies of either ice or quartz, nor in any of the computer simulations of fabrics<sup>13,14</sup>. The displacement of strain markers between layer boundaries, the variable axial-surface orientations associated with the minor folds, and their partial parallelism to the layer boundaries, suggest that during the deformation there has been some degree of layer boundary slip. Therefore buckling and flattening proceed simultaneously with layer boundary slip and a component of plane strain. This compares markedly with the isotropic samples where the deformation is strictly a homogeneous plane-strain deformation. It is the layering then that is crucial in determining the deformation path of any point in the material.



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## Thiocyanate in Red Sea brine and its implications

THE Oparin–Haldane hypothesis about the origin of life has provided a rational theoretical model for laboratory research. Almost thirty years of experiments have supported this hypothesis. Verification of the hypothesis in terrestrial environments, however, has not been documented. Some environments such as thermal springs in the Kuril Islands have been reported to have possible chemical precursors but lack of sterility and other factors have not made it possible to establish evidence for natural, terrestrial abiotic synthesis<sup>1</sup>. We identify here the Atlantis II Deep brine as a promising site for searching for chemical precursors, report finding thiocyanate in the brine and suggest a relationship between chemical evolution and the evolution of the Earth's crust.

We have recently suggested<sup>2</sup> that the Red Sea brines, located at the bottom of the sea in the rift valley of an active axis of spreading global plates, might be a fruitful place to search for terrestrial evidence of abiotic synthesis of life precursors<sup>2</sup>. The Atlantis II Deep brine (21°22' N, 38°05' E) was thought to be especially promising because: (1) it has been reported sterile<sup>3</sup>; (2) it has a methane enrichment 10<sup>3</sup> times normal seawater<sup>4</sup>; (3) it has no free oxygen (O<sub>2</sub>)<sup>5</sup>; (4) it is unusually warm (63 °C) (D. A. Ross, personal communication); and (5) it has been reported as a 'reducing' environment<sup>5</sup>.

During Cruise 93, Leg 19 of the RV Atlantis II in spring 1977 the Atlantis II Deep brine was identified by seismic profiling and depth recording revealing a brine of about 200 m thickness at a depth of 2,000 m. Water samples were collected using 10 l and 30 l Niskin samplers set at 7 m intervals from 14 m above the bottom to 225 m above the bottom at Station 87. Our samples were subsequently stored in sealed, sterilised, brown, opaque Nalgene bottles and thymol crystals were added to half to ensure continuous sterility. Sterility of the samples taken at this station from the brine has been confirmed (R. Cuhl, personal communication).

Aliquots (5 cm<sup>3</sup>) of Bottle no. 1 (30 l) from Station 87 were chromatographed using butanol/ammonia/water on Whatman no. 1 paper<sup>7</sup>. The *R<sub>f</sub>* of known thiocyanate was 0.25 in these conditions. Strips of 4 cm length corresponding to the *R<sub>f</sub>* of known thiocyanate were cut, eluted with 5 cm<sup>3</sup> of distilled water and flash evaporated to their original volume. Aliquots of 0.5 cm<sup>3</sup> were tested for thiocyanate using 0.3 cm<sup>3</sup> 0.1 M cupric chloride solution and 0.5 cm<sup>3</sup> of a ferric nitrate/nitric acid reagent. Thiocyanate produces a wine red colour with these reagents and a spectrophotometric absorbance peak at 4,600 Å

(ref. 8). (This analytical method was developed to measure thiosulphate by adding cyanide to produce sulphite and thiocyanate. The reagents are added to produce a ferric thiocyanate complex. The thiosulphate concentration corresponds to the absorption of the complex. We used this method as a direct measure of thiocyanate. Thiosulphate without cyanide produces no spectrophotometric absorbance with these reagents.)

The Atlantis II Deep brine samples were analysed against a 'null' brine of 5M NaCl, 0.2M KBr and 0.02M KHCO<sub>3</sub> on a Zeiss ZFM 4/v4QIII/PMQII spectrophotometric system and a Unicam SP80 recording spectrophotometer. The spectra of the samples were scanned from 4,300 Å to 6,000 Å and compared with the spectrum of known ferric thiocyanate complexes with respect to general shape and the absorption ratios at several wavelengths. Aliquots (5 cm<sup>3</sup>) were chromatographed, eluted and concentrated to one-tenth the original volume and analysed. Results of the direct analysis confirm a thiocyanate concentration of 2.4 × 10<sup>-5</sup> M (mean of 12 spectrophotometric analyses; standard deviation = 1.2) in the Atlantis II Deep brine. No thiocyanate was detected in a control sample of Red Sea water.

In four experiments, a known amount of thiocyanate was added to the brine to determine whether this would increase the spectrophotometric absorption corresponding to the amount added. This was confirmed in all four experiments. Results of these experiments yield a mean thiocyanate concentration of 2.8 × 10<sup>-5</sup> M (range 2.3–4.0 × 10<sup>-5</sup> M) in the brine. This concentration is significant from the point of view of prebiotic chemistry.

Some samples were also treated with Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> before analysis to test for the presence of cyanide ions<sup>8</sup> but none were found. All cyanide, however, would be expected to convert to thiocyanate since the underlying sediments through which the brine flows are rich in elemental sulphur and sulphides such as pyrite, chalcocopyrite, sphalerite and marcasite<sup>9,10</sup> making the environment similar to conditions for the commercial preparation of thiocyanate from cyanide.

What is the origin of the thiocyanate in the Atlantis II Deep brine? No marine organisms are known to release cyanide or thiocyanate into the marine environment. Both cyanide and thiocyanate have been reported from geothermal springs<sup>1</sup>. Hydrogen cyanide may have been formed abiotically and released from within the ridge system after which it reacted with sulphur or sulphides to form thiocyanate. We think this is the most likely source of the thiocyanate.

The important role of hydrogen cyanide and its related chemical species in the prebiotic synthesis of purines, pyrimidines and amino acids is well-documented from Miller<sup>11</sup> to Ferris and Joshi<sup>12</sup>. The discovery of thiocyanate in a sterile, natural terrestrial environment such as the Atlantis II Deep brine is consistent with our suggestion that there may be a relationship between the evolution of the Earth's crust at spreading axes and the origin of life. While the presence of thiocyanate in a sterile brine does not equivocally demonstrate the abiotic origin of the cyanide from which the thiocyanate may derive, it suggests that further analysis of the Atlantis II Deep brine should be undertaken to assess the character of organic molecules present. Previous analysis of hydrocarbons in the brine has indicated pronounced enrichment in methane, ethane and low molecular weight paraffins<sup>3</sup>. Abiotic synthesis of amino acids in this environment is plausible.

Criteria for determining whether amino acids were formed biotically or abiotically include the D and L enantiomer ratio, the <sup>13</sup>C and <sup>12</sup>C ratio and the type of amino acids present<sup>13</sup>. As the brine has a high temperature, the D and L enantiomer ratio may not be a useful criterion. Amino acids such as α-aminoisobutyric acid, α-amino-n-butyric acid, N-ethylglycine, N-methylalanine, norvaline and sarcosine in amounts similar to those found on the Murchison meteorite would suggest an abiotic origin. We intend to search for amino acids in the Atlantis II Deep brine and will use these criteria in our evaluation.



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## Origin of carbonatites by liquid immiscibility

THE existence of carbonatite magmas has been generally accepted<sup>1</sup>, but their origin remains uncertain. The more favoured petrogenetic models include: (1) direct partial melting of the upper mantle<sup>2–5</sup>; (2) fractional crystallisation of CO<sub>2</sub>-rich alkaline silicate magma<sup>6</sup>; and (3) separation of an immiscible carbonate melt from an initially homogeneous CO<sub>2</sub>-rich alkaline silicate magma<sup>7–10</sup>. Experiments have shown all of these processes to be feasible<sup>5–7</sup>, and each may generate the geochemical characteristics of carbonatite, such as enrichment in rare earths and other incompatible trace and minor elements<sup>11,12</sup>, and low <sup>87</sup>Sr/<sup>86</sup>Sr ratios<sup>13</sup>. Here we discuss the role of immiscibility, and report new experimental data which demonstrate for the first time that liquid immiscibility does occur between silicate and carbonate liquids of the compositions found in nature.

While previous experimental work suggests that high concentrations of Na<sub>2</sub>O are required for immiscibility<sup>7,14,15</sup>, the great majority of analysed carbonatites are rich in CaO with Na<sub>2</sub>O as only a minor constituent, seemingly ruling out a role for immiscibility in their genesis (for example, mean of 12 Kenyan alvikites CaO = 50.83, Na<sub>2</sub>O = 0.48 wt % ref. 9, p. 317). However, it has been argued that the present-day compositions of most carbonatites are not representative of the original magmas but have lost large quantities of alkali oxides during fenitisation and due to leaching by meteoric waters<sup>2,3,9,16,17</sup>. This view is supported by the study of inclusions in minerals from carbonatites, which show that alkaline CO<sub>2</sub>-rich fluids were present at high temperatures<sup>18–20</sup>. It is significant that the only carbonatite which has been observed at the time of eruption, the natrocarbonatite of Oldoinyo Lengai, Tanzania<sup>21–25</sup>, contains about 30 wt % Na<sub>2</sub>O (Table 1). This lava has retained its alkalis



**Fig. 1** Spheres of carbonate in silicate glass. The diameters range up to about 2 mm. The small pits seen in the carbonate are probably CO<sub>2</sub> vesicles. Starting material was 15% carbonate plus 85% nephelinite, run conditions 2 kbar, 1,000 °C for 18 h.

because it reached the surface and quenched, precluding fenitisation processes, and because it was sampled before it had been leached. Xenoliths of plutonic carbonatite from the same volcano are CaCO<sub>3</sub>-rich sövite<sup>24</sup>. The main aim of the present study was to investigate the possibility of an immiscibility relationship between the Oldoinyo Lengai natrocarbonatite and the contemporaneous silicate magmas.

Two synthetic carbonatite compositions were made up, one a simplified version of the Oldoinyo Lengai lava, and the other richer in CaCO<sub>3</sub> (Table 1). These were mixed and ground in various proportions with two powdered silicate lavas from Oldoinyo Lengai, a phonolite and a nephelinite (Table 1). About 0.7 g samples of dried powder were sealed in noble-metal tubes and run at pressures from 0.7 to 7.6 kbar and temperatures from 900 to 1,250 °C in argon-filled internally heated pressure vessels. Selected run data are given in Table 1.

In general, charges which were not of extreme composition (that is, very close to either silicate or carbonate starting materials) showed characteristics on quenching consistent with the coexistence of two melts at the pressure and temperature of the experiment. Silicate melts quenched to glasses, which were separated by smooth, sharp menisci from carbonate melts, which quenched to crystalline aggregates. The actual geometry of the association depended on the proportion of carbonate in the starting mixture, and the temperature and pressure of the run. Where the proportion of silicate greatly exceeded that of carbonate, globules of carbonate were observed in silicate glass, see, for example, Fig. 1. Where there was a moderate to high proportion of carbonate, however, (≥40 wt %), a central ovoid slug of silicate glass normally occurred, flanked by ends of carbonate. Depending on temperature, a small number of globules of one phase was observed in the other, but at temperatures ≥1,000 °C almost complete separation of melts took place.

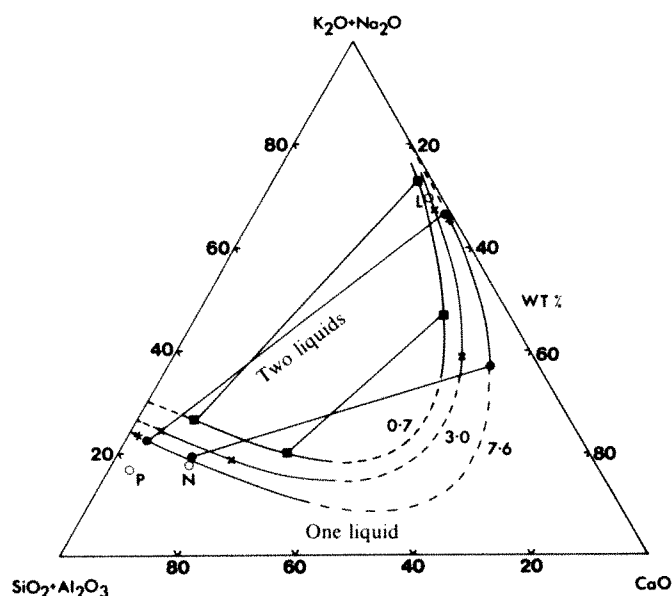
Where a significant amount of crystals are present in the silicate melt the physical separation of the two melts is not so perfect. In 50/50 original mixtures of silicate and carbonate the final geometry of the charge was still a central lozenge of silicate glass with two end cappings of carbonate but significant amounts of carbonate globules occurred throughout the silicate glass. The larger volumes of carbonate remain essentially silicate-free, however, and it was easy to hand pick a silicate-free carbonate fraction. This was analysed by wet chemical methods. The silicate glass was analysed by electron microprobe. Microprobe



analysis of the carbonate fraction using a defocused beam generally gave broadly comparable results with the wet chemical analysis, but was considered less reliable because of the instability of the carbonates. However, it did allow some supplementary data to be obtained on elements which were not analysed wet chemically.

Several features suggest that the phases we have analysed represent equilibrium immiscible melts and are not just the result of the failure of the starting mixtures to homogenise. These include: (1) scanning electron microscope study of the charges shows that the meniscus between the melts is sharp on a submicron scale; (2) the silicate glasses are compositionally homogeneous throughout the slugs; (3) spheroids of silicate glass, ~100  $\mu\text{m}$  diameter, in the quenched carbonate melts have compositions identical to those of the main silicate slugs; (4) spheroids of quenched carbonate liquid in the silicate glass, a few tens of  $\mu\text{m}$  diameter, yield microprobe analyses which are comparable to the wet chemical analyses of the main carbonate fraction, bearing in mind their instability in the electron beam. We have also carried out reversal type experiments to test for gross metastability. In the first instance, two melts were held at conditions where the miscibility gap is narrow (high  $T$ , low  $P$ ), so that the silicate dissolved large amounts of carbonate. The homogeneous silicate-glass fraction was removed and re-run in conditions where the miscibility gap is wide (low  $T$ , high  $P$ ) and it exsolved small spheres of carbonate. Second, it was found that silicate glass containing a few spheroids of carbonate (~0.1 mm diameter), produced at high  $P$  and low  $T$  could be homogenised by re-running at low  $P$  and high  $T$ . These experiments demonstrate that the carbonate melt would readily dissolve, or exsolve from the silicate melt in the conditions of interest in this study.

The main characteristics of the immiscibility field encountered in this study are shown in Table 1 and Fig. 2. The components of Fig. 2,  $\text{SiO}_2 + \text{Al}_2\text{O}_3$ ,  $\text{Na}_2\text{O} + \text{K}_2\text{O}$ ,  $\text{CaO}$ , represent ~90% of the  $\text{CO}_2$ -free portions of both melts. Most of the data are for one temperature, 1,100 °C, but for three pressures, 0.7, 3.0 and 7.6 kbar. Conjugate melts at both 0.7 and



**Fig. 2** Miscibility gap between carbonate and silicate melts. Conjugate melts are shown for 0.7 kbar, 1,100 °C (■); 3.0 kbar, 1,100 °C (×); 7.6 kbar, 1,100 °C (●); and 3.0 kbar, 900 °C (\*). The boundary between the one-liquid and two-liquid fields has been drawn for each pressure and 1,100 °C. Conjugate liquids at 0.7 kbar and 7.6 kbar are joined by tie-lines. The liquids furthest from the CaO apex were produced from mixtures of phonolite and CaO-poor carbonatite, those closer to the CaO apex were produced from mixtures of nephelinite and CaO-rich carbonatite. P, Oldoinyo Lengai phonolite; N, nephelinite; L, natrocarbonatite.

7.6 kbar are joined by tie-lines. The data points for each pressure are joined by curves which represent the isothermal, isobaric phase boundaries between the one-liquid and two-liquid fields (Fig. 2). The data indicate that the miscibility gap closes towards the CaO apex; this is supported by data on other systems, for example, immiscibility was not found in  $\text{NaAlSi}_3\text{O}_8\text{-CaCO}_3\text{-H}_2\text{O}$  at 1 kbar (ref. 6). Therefore, we have extrapolated the field boundaries and shown the closure as dashed lines (Fig. 2). Comparison of the boundary curves shows that increasing pressure expands the miscibility gap. The inclusion on Fig. 2 of a run at 3 kbar and 900 °C shows that decreasing temperature expands the two-liquid field in a manner analogous to increasing pressure. It is stressed, however, that the effect of changing  $P$  or  $T$  is not just a simple narrowing or widening of the gap but complicated changes in composition take place including rotation of the tie-lines.

The compositions of Oldoinyo Lengai phonolite (P), nephelinite (N) and carbonatite (L) are also shown in Fig. 2. The position of the tie-lines suggest that the natrocarbonatite is likely to have been immiscible with a phonolitic magma at high pressures and temperatures. Nephelinite melt, on the other hand, was consistently found to be immiscible with carbonate melts much richer in CaO than the Oldoinyo Lengai lava (Table 1, Fig. 2). Therefore the experimental study supports the view<sup>25</sup> that the Oldoinyo Lengai carbonatite exsolved from a  $\text{CO}_2$ -rich phonolitic magma.

Most natural carbonatite compositions lie close to the CaO apex of Fig. 2, and it seems unlikely that the tie-lines could rotate sufficiently for phonolitic or nephelinitic magmas to be conjugate with carbonate melts so poor in  $\text{Na}_2\text{O}$ , even at very high pressures. If these carbonatites did originate by immiscibility, then significant loss of alkalis must have occurred at some stage in their development, as proposed above. The failure of many experimentalists to observe immiscibility during experiments on  $\text{CO}_2$ -rich systems at pressures to 30 kbar<sup>6,14,15</sup>, or to confirm the presence of immiscibility which had been tentatively identified<sup>26,27</sup>, is because the compositions studied were poor in

**Table 1** Starting materials and run products

	1	2	3	4	5	6	7	8
$\text{SiO}_2$	41.93	2.00	44.46	4.5	52.88	—	52.23	0.16
$\text{TiO}_2$	0.92	—	0.73	NA	0.93	—	0.71	0.18
$\text{Al}_2\text{O}_3$	15.55	—	14.68	0.40	19.89	—	17.41	0.5
$\text{Fe}_2\text{O}_3$	6.41	—	—	—	4.07	—	—	—
$\text{FeO}$	2.28	—	5.41*	2.30*	1.58	—	2.99*	1.54*
$\text{MgO}$	1.28	1.00	1.05	0.92	0.45	—	b.d.	0.76
$\text{CaO}$	10.89	33.32	11.13	31.59	2.67	16.69	1.96	21.82
$\text{Na}_2\text{O}$	10.26	16.66	11.07	16.35	10.63	33.38	13.28	27.00
$\text{K}_2\text{O}$	4.95	5.55	5.73	5.16	4.91	8.34	6.95	5.53
$\text{P}_2\text{O}_5$	0.54	1.00	0.23	1.21	0.11	1.00	b.d.	1.01
$\text{CO}_2$	2.39	35.47	NA	NA	0.15	35.59	NA	NA
F	NA	2.00	NA	NA	NA	2.00	NA	NA
Cl	NA	3.00	0.48	NA	NA	3.00	0.19	NA
Others	2.28	—	—	—	1.77	—	—	—
Totals	99.68	100.00	94.97	62.44	100.04	100.00	96.72	58.50

1, Nephelinite BD119, Oldoinyo Lengai. Analysis includes  $\text{H}_2\text{O}^+ = 0.80$ ,  $\text{H}_2\text{O}^- = 0.85$ .

2, Synthetic high- $\text{CaCO}_3$  carbonatite.

3, Immiscible silicate melt produced by melting equal weight mixtures of 1 and 2 at 7.6 kbar, 1,100 °C. (● in Fig. 2.)

4, Carbonate melt in equilibrium with 3. (● in Fig. 2.)

5, Phonolite BD50, Oldoinyo Lengai.

6, Synthetic carbonatite, a simplified mean of Oldoinyo Lengai lavas, with  $\text{Na}_2\text{O}:\text{CaO}:\text{K}_2\text{O} = 4:2:1$ .

7, Immiscible silicate melt produced by melting equal weight mixtures of 5 and 6 at 3 kbar, 900 °C. (\* in Fig. 2.)

8, Carbonate melt in equilibrium with 7. (\* in Fig. 2.)

NA, not analysed; b.d., below detection limits of energy dispersive microprobe (~0.3 wt %).

Analysts: T. Jensen, carbonate run products, I. Freestone, silicate run products, D. G. Powell, lavas.

\* Total iron as FeO.

Na<sub>2</sub>O, and plotted in the one-liquid field of Fig. 2. However, the compositions of many mafic and ultramafic alkaline igneous rocks, such as melanephelinites, melilitites, kimberlites and alnoites, also project into this field, suggesting that the corresponding magmas are unlikely to exsolve carbonate melts at crustal pressures, although immiscibility in the mantle remains a possibility.

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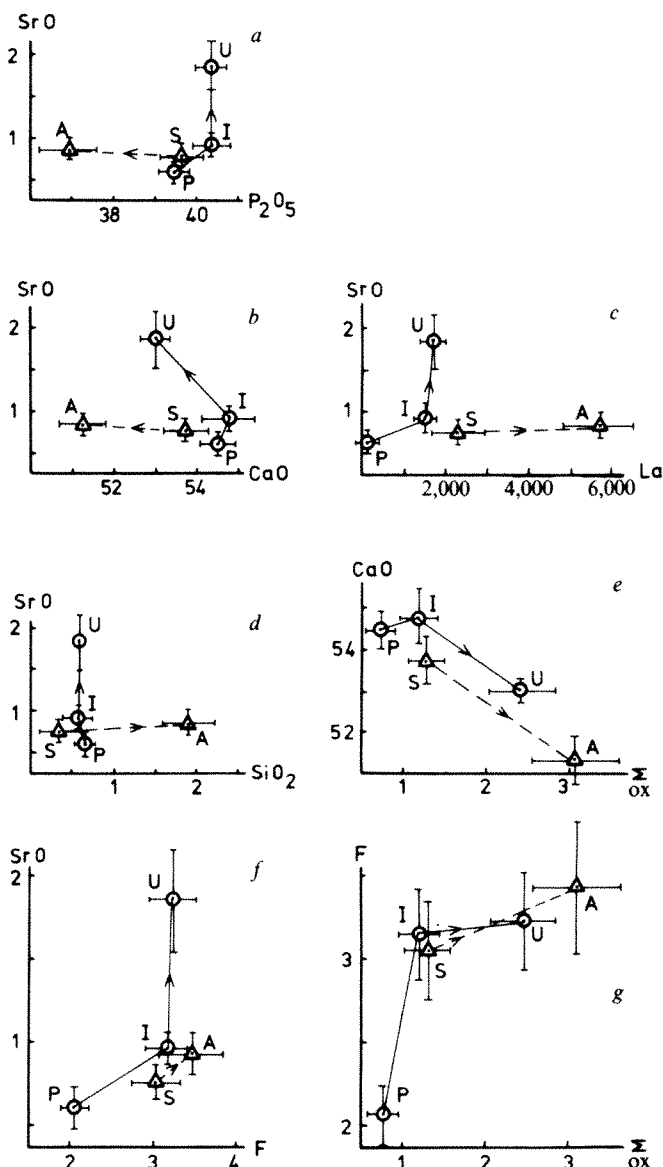
## Variation in apatite composition in ijolitic and carbonatitic igneous rocks

WE present here evidence that apatite crystals formed in alkaline magmas during the early stages of both the ijolitic (pyroxenite → ijolite → urtite) and carbonatitic (sövite → alvikite) differentiation series<sup>1</sup> have similar chemical compositions, but that with subsequent crystal differentiation in both series, the compositions of the apatites can change progressively along two separate paths. The evidence of a bifurcating pattern of apatite compositions from the various rock types studied is not in accord with the theory<sup>2,3</sup> that carbonatites are the ultimate product of fractional crystallisation of the ijolitic series. The evidence suggests that ijolite and sövite are the crystalline products of conjugate immiscible liquids.

Any wet chemical method of apatite analysis relying on mineral separation techniques introduces the possibility of contamination<sup>4</sup>, and hence many published apatite analyses must be considered suspect. Electron microprobe (EMP) analysis, however, can provide good quality data, and minor chemical variations can be used as reliable indicators of fractionation processes<sup>5</sup>. Our data show greater variation in chemical composition than was previously indicated<sup>6</sup>.

The means of 115 EMP analyses of apatites in Table 1 are taken from five associated rock types from two adjacent well documented strongly alkaline igneous complexes<sup>1</sup>. Very similar analytical data have been obtained from other igneous and sub-volcanic complexes in this west Kenyan-east Ugandan-north Tanzanian Tertiary magmatic province. It seems that the apatites from any one particular rock type and texture all have the same chemical composition, no matter which igneous complex they are taken from in the province. Apatites, like other igneous minerals, seem to reflect chemically the nature of the liquids from which they were precipitated.

The five rock types mentioned under Table 1 provide good representatives of the ijolitic and the carbonatitic differentiation



**Fig. 1** Variation diagram for apatites from ijolitic (—○—) and carbonatitic (—△—) rocks. Oxides and fluorine plotted in wt %: La in p.p.m. Σ<sub>ox</sub> is sum of Na, Ce, La, Sr oxides. Arrows show direction of differentiation. P, pyroxenite; I, ijolite; U, urtite; S, sövite; A, alvikite.

Table 1 EMP analyses of apatites (means)

	1 (n = 20)		2 (n = 33)		3 (n = 5)		4 (n = 30)		5 (n = 24)	
	wt %	s.d.	wt %	s.d.	wt %	s.d.	wt %	s.d.	wt %	s.d.
SiO <sub>2</sub>	0.65	0.08	0.56	0.18	0.60	0.05	0.30	0.15	1.91	0.37
P <sub>2</sub> O <sub>5</sub>	39.46	0.30	40.43	0.50	40.38	0.29	39.75	0.55	36.97	0.70
CaO	54.50	0.41	54.84	0.64	53.00	0.16	53.78	0.55	51.34	0.50
SrO	0.61	0.10	0.89	0.13	1.85	0.37	0.74	0.09	0.83	0.11
Ce <sub>2</sub> O <sub>3</sub>	0.03	0.03	0.12	0.02	0.35	0.04	0.21	0.06	1.49	0.20
La <sub>2</sub> O <sub>3</sub>	0.01	0.01	0.17	0.02	0.20	0.02	0.27	0.07	0.67	0.09
Na <sub>2</sub> O	0.08	0.03	0.04	0.06	0.04	0.01	0.10	0.03	0.10	0.15
F	2.07	0.19	3.16	0.27	3.23	0.29	3.06	0.32	3.42	0.41
	97.41		100.21		99.65		98.21		96.73	
less O = F	0.87		1.33		1.36		1.29		1.44	
	96.54		98.88		98.29		96.92		95.29	

Analyses were carried out on a Cambridge Mark V Electron Micro-Probe Analyser at 15 kV and specimen current of 20 nA and counts obtained were corrected for dead-time and ZAF using a modified MAGIC IV program.

1 From alkali pyroxenite, Usaki, West Kenya (U 1122).

2 From ijolite, Usaki, West Kenya (U 1256).

3 From urtite, Usaki, West Kenya (U 79).

4 From sövite, North Ruri, West Kenya (N 43).

5 From alvikite, North Ruri, West Kenya (N 179).

series. The pyroxenite is a product from an earlier partly cumulative stage in the ijolitic differentiation series than is represented by ijolite. The ijolite (comprising mainly nepheline and aegirine-augite) is the approximate plutonic equivalent of a nephelinitic magma, whilst the urtite (largely composed of nepheline) is the fractionation product. These three represent a typical sequence of the ijolitic magmatic differentiation series as recognised at most ijolite intrusive complexes<sup>1,3</sup>. The sövite is the earliest and main member of the carbonatite series, and the alvikite is the second member of the series derived from the sövite by magmatic differentiation<sup>1,7</sup>.

The structural formulae of the five mean apatite analyses (Table 2) are calculated on the basis of 10 ions in the calcium position<sup>8</sup> rather than to 26 anions. This allows an estimate to be made, by difference, of the carbonate content of the apatite, based on the assumption that CO<sub>3</sub> groups substitute for PO<sub>4</sub> groups in the lattice<sup>9</sup>.

The substitution inferred of P by Si in notable amounts is real because most of the apatite crystals analysed from silicate rocks were single crystals mounted in resin, and that the apatite crystals were checked for lack of inclusions such as sometimes exist in these crystals<sup>10</sup>. The increasing proportion of Si with differentiation in the carbonatitic apatites is in keeping with the fact that free silica is commonly developed in late-stage carbonatites<sup>1</sup>. The reliability of the estimate of the hydroxyl content is less certain, although the F determinations in Table 1 have a good precision and Cl was below the detection limit (100 p.p.m.). The elements Mg, Fe and Mn were also sought but were below the minimum detection limit (all near 200 p.p.m.), as was Ba (500 p.p.m.).

The distinctions between the silicate and carbonate differentiation series, which are reflected in the chemical variations of the apatites, are best seen in Fig. 1. Figure 1a-d show the divergence of the two trends, e and g show that grouping of some elements produces parallel trends but still shows the proposed common parentage, and f shows that the apatites from the silicate rocks can exhibit more extreme fractionation than those from the carbonatites: a situation which could not arise if the carbonatites were a fractionational crystallisation product of the ijolitic magma. Two further significant features of all the plots in Fig. 1 are that both trends have a common starting position, and that this position is near that for ijolite and sövite which are themselves considered to be the sub-volcanic materials most closely related to the parental compositions of the two differentiation series.

The close similarity in composition of the apatites from both ijolite and sövite shows that the relationship between the two liquids from which these two rocks crystallised could have been that of a pair of liquids in equilibrium with each other. It has already been suggested that the ijolitic and carbonatitic rock series are related by liquid immiscibility<sup>1,11</sup> but, as far as we know, this is the first time that liquid immiscibility has been shown to be possible in natural alkaline igneous rocks based on the occurrence of one mineral phase, apatite, being in equilibrium with two liquid phases. This test for liquid immiscibility was recognised by Bowen<sup>12</sup> when he stated "Not only are they (the immiscible pair of liquids) in equilibrium with each other but they both must be in equilibrium with any additional phase".

The variable distribution of major and minor elements in apatites within ijolitic and carbonatitic alkaline igneous rocks

Table 2 Ions on basis 10 (Ca, REE, Sr, Na) for apatite analyses in Table 1

	1	2	3	4	5
Si	0.11	0.09	0.10	0.05	0.34
P	5.67	5.76	5.87	5.76	5.53
Ca	9.92	9.89	9.77	9.87	9.74
Sr	0.06	0.09	0.19	0.07	0.09
Ce	0.00	0.00	0.02	0.01	0.10
La	0.00	0.01	0.01	0.02	0.04
Na	0.02	0.01	0.01	0.03	0.03
F	1.11	1.68	1.76	1.65	1.91
(CO <sub>3</sub> )	0.22	0.15	0.03	0.19	0.13
(OH)	0.89	0.32	0.24	0.35	0.09
(O, OH, F)	26.39	26.58	26.88	26.52	26.56



show that their respective differentiation trends follow two independent paths, both emanating from magmatic compositions which could have been in equilibrium with each other. This relationship is consistent with liquid immiscibility rather than crystal fractionation as the governing petrogenetic process.

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## First results from the Reykjanes Ridge Iceland Seismic Project 1977

PALAEOGEOGRAPHIC reconstructions of the North Atlantic area, based on plate tectonic principles<sup>1-3</sup>, suggest that Iceland is of pure oceanic origin as Greenland and Europe (including Rockall Plateau) seem to have been closely connected before 56 Myr BP (magnetic lineation 24). Oceanic origin is also suggested by the location of Iceland astride the axis of the Mid-Atlantic Ridge, by its surface geology and by the young age (16 Myr) of its oldest rocks. Further evidence comes from refraction seismic investigations<sup>4</sup> yielding a layering of the Icelandic crust resembling the typical oceanic crust in velocity values, but with greater thickness of individual layers. A remarkable result was the discovery of a layer with *P*-wave velocities close to 7.2 km s<sup>-1</sup> at a depth of 8–15 km, which was interpreted as the top of an anomalous upper mantle below Iceland. According to studies of teleseismic travel time residuals<sup>5,6</sup>, as well as apparent velocity measurements of *P*-arrivals from Mid-Atlantic Ridge earthquakes<sup>7</sup>, this anomalous mantle may extend to 240 km depth. A very different model, however, has been derived from more recent long range refraction seismic measurements<sup>8</sup>. It is characterised by a more or less continental deeper crust and a normal upper mantle with a *P*-wave velocity of 8.0 km s<sup>-1</sup> at a depth of 50–60 km below Iceland. This model could also account for the low apparent velocities given by Francis<sup>7</sup> and, if true, would require drastic modifications of current ideas of seafloor spreading. It has been used as an argument against the possibility of any significant amount of drift at the latitude of Iceland<sup>9</sup>. Geophysical evidence from Reykjanes and Kolbeinsey Ridges<sup>10,11</sup>, on the other hand, strongly favours an oceanic origin of the surroundings of Iceland. Earlier refraction seismic measurements on the crest and western flank of the Reykjanes Ridge<sup>12,13</sup> indicate a systematic change of crustal structure with age and a crustal structure typical of oceanic basins has been found some 200 km south-east of the ridge<sup>14</sup>. All the marine refraction work so far has shown anomalously low mantle velocities (7.2–7.8 km s<sup>-1</sup>) but very little information is available on the structure of the oceanic upper mantle itself because of the limited range of the seismic

profiles. In 1977 an 800-km long seismic refraction profile was shot across Iceland and along the southeastern flank of the Reykjanes Ridge (Fig. 1). The main purpose of the experiment was to resolve the structure of the crust and upper mantle to greater depth than previously possible and to study the transition from the oceanic to the Icelandic structure. This experiment was planned and carried out by an international working group (Reykjanes Ridge Iceland Seismic Project, RRISP).

The experiment described here was a joint land-sea project with a number of shotpoints on sea and land giving a set of reversed and overlapping profile segments and large observational distances. Figure 1 shows positions of shots and receivers. Nineteen shots with charges from 0.4 to 4.0 tons were fired and recorded on land by up to 42 mobile stations of MARS-66 type<sup>15</sup> from Germany, 12 automatic stations from the Soviet Union and 35 direct recording stations of the Icelandic short period seismic network. The shots at A to F were recorded by the MARS-66 stations on the main line running from Heimaey at the southern coast mostly through the neo-volcanic zone towards the north-east. Shots G and H were observed along the southeastern coast to give a reference profile in the older part of Iceland. In the marine part ocean bottom seismographs<sup>16</sup> and anchored telemetric buoy systems with ground hydrophones<sup>17</sup> recorded series of smaller shots (50–250 kg). The shots were spaced 1.8 km apart and could be recorded to 150 km range. In addition to the 800-km long main line observed 110 km east of the ridge crest three more refraction lines were observed at sea, one parallel and closer to the ridge axis and two perpendicular to it. Most of the marine profiles were covered also by sparker, gravity, total magnetic field and narrow beam echo sounder measurements.

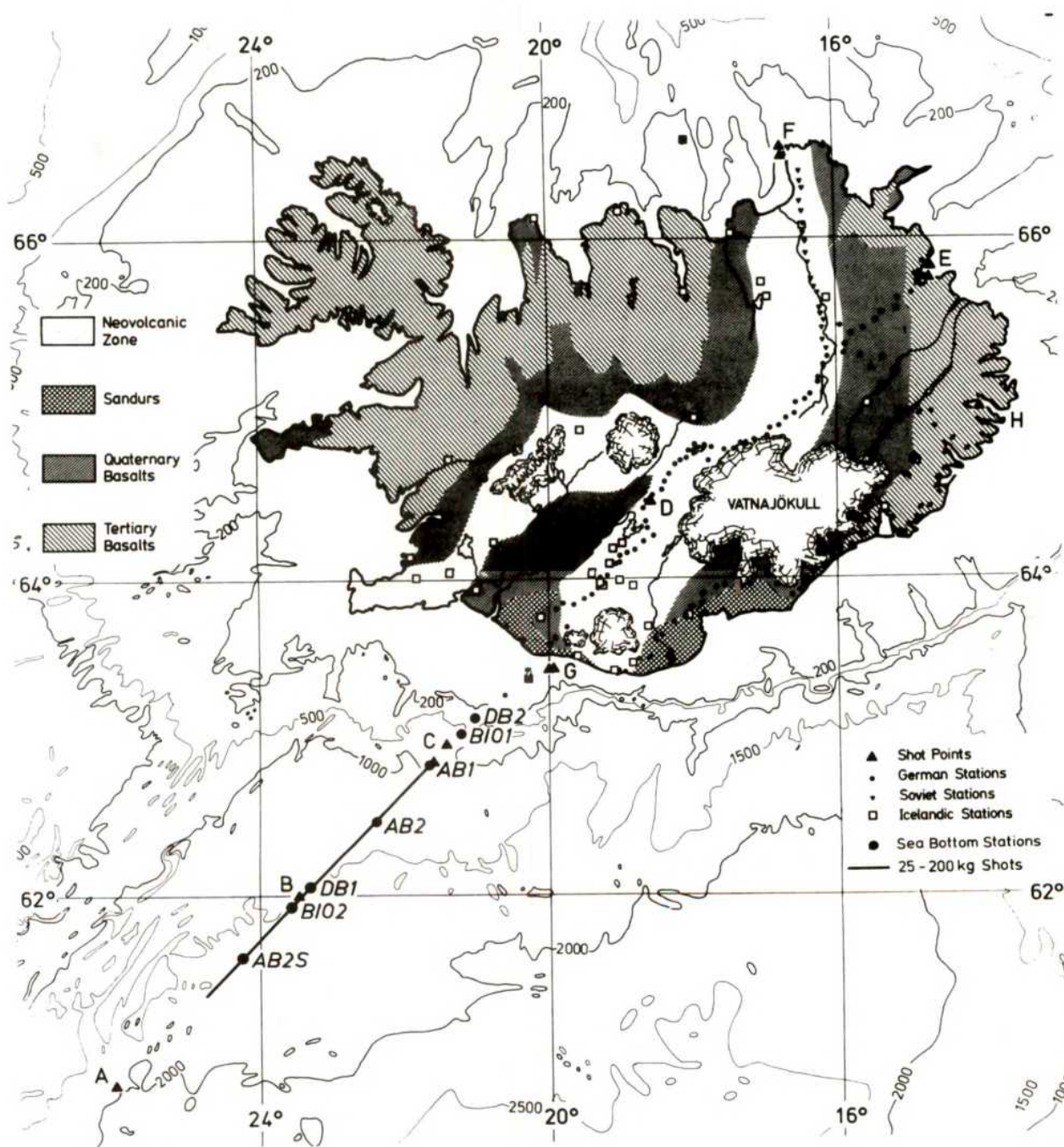
We shall concentrate on the results obtained on the main line. Figures 2 and 3 are examples of a marine and a land profile segment respectively. The upper part of Fig. 2 shows the record section (reduction velocity is 6 km s<sup>-1</sup>) obtained by shooting into the bottom hydrophone of buoy AB1 from the south-west. First arrivals can be fitted by three straight lines indicating a well defined layering of the crust. From this and the reversed line we obtain the model shown in the lower part of Fig. 2. Apart from small dips of the sea bottom and basement the layers are horizontal. The velocities and layer thicknesses are within the range of typical oceanic values. It is not known whether the velocity of 7.7 km s<sup>-1</sup> represents anomalous mantle or crustal layer 3b. From the records of OBS BI01, however, which show first arrivals with apparent velocities of 8.3 km s<sup>-1</sup> at distances greater than 90 km, it is evident that normal upper mantle is present at greater depth.

The upper part of Fig. 3 shows a typical land record section. It was obtained for shotpoint E and reduced by 7 km s<sup>-1</sup>. The record sections for shots D, F, G and H look very similar. There are distinct differences in travel time and apparent velocity within the first 100 km reflecting crustal heterogeneity but apparent velocities at greater distances are rather uniform and close to 7.0 km s<sup>-1</sup>. Clear first arrivals can be traced up to distances of 300 km, whereas later arrivals are difficult to correlate. Wave groups with apparent velocities around 8 km s<sup>-1</sup> which would indicate a normal upper mantle below Iceland can not be found. This holds also for records of the more distant shots A and B. In spite of charges of 4 and 2 tons, fired from RV Meteor in optimum water depth, only weak first arrivals were obtained from these shots at the recording sites in Iceland. As far as correlation of these arrivals is possible, again apparent velocities only slightly greater than 7.0 km s<sup>-1</sup> are obtained. On the other hand, if apparent velocities between shotpoints A, B and C are calculated for individual stations in Iceland one obtains values as high as 8.4 or even 9.0 km s<sup>-1</sup>. This is further evidence that, contrary to Iceland, the 10 Myr old Reykjanes Ridge on the seaward continuation of the profile has a well developed lithosphere. From the records of shot C, which give apparent velocities close to 8.0 km s<sup>-1</sup> for some stations near the south coast but 7.0 km s<sup>-1</sup> for more distant stations, it becomes evident that the transition from the normal lithosphere of the

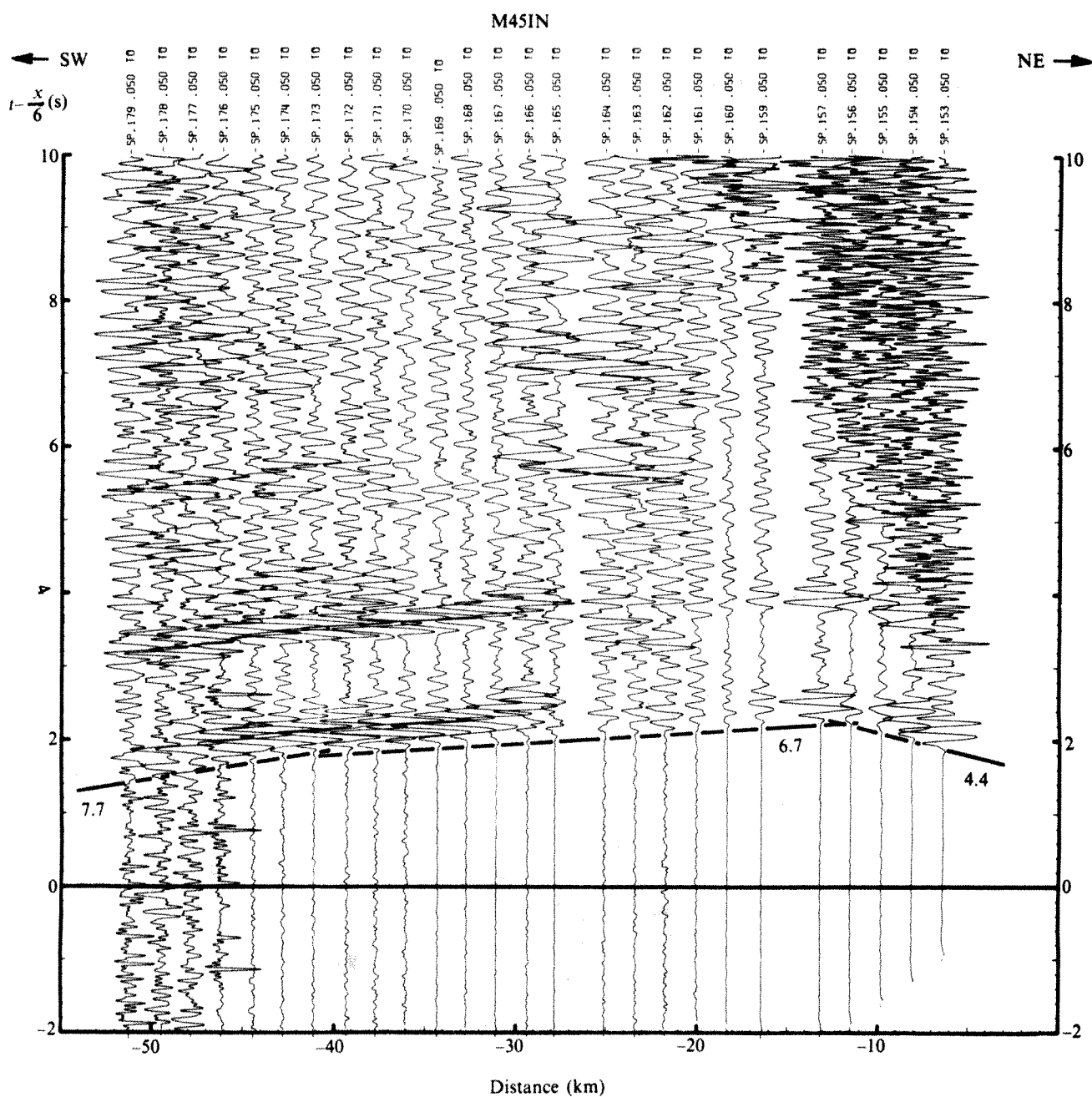
Reykjanes Ridge to the anomalous deep structure of Iceland takes place near the southern shelf edge. The lower part of Fig. 3 shows our latest model with the corresponding ray paths for shotpoint E as calculated by a ray tracing method<sup>18</sup>. The calculated travel time curve has been superimposed on the record section and fits the observed arrivals well. Although the evaluation is still in progress and modifications of the model are to be expected, we feel confident with the conclusion that the P-wave velocity of  $7.0 \text{ km s}^{-1}$  is reached below Iceland in 10–15 km depth. There is no room for a 30-km thick layer with velocities from 6.5 to  $7.0 \text{ km s}^{-1}$  which could be interpreted as continental. In depths below 10–15 km the velocity increases only slightly

and velocity reversals cannot be ruled out. This anomalous layer must extend to depths greater than 60 km, as a normal upper mantle at 50–60 km depth<sup>8</sup> would produce travel time segments with apparent velocities close to  $8 \text{ km s}^{-1}$ , which have not been observed in the record sections.

On the other hand, it is known from surface wave studies<sup>19</sup> that the top of the asthenosphere is to be expected at about 60 km depth below the flank of the Reykjanes Ridge. It seems therefore most natural to interpret the anomalous layer below Iceland as a diapiric updoming of the asthenosphere and the term 'anomalous mantle' seems quite adequate. Further evidence for this interpretation comes from the observation of



**Fig. 1** Simplified geological map of Iceland and bathymetric chart of the surrounding ocean showing shotpoints and recording sites of the RRISP 77 project.



**Fig. 2** Record section of the line AB1-AB2 recorded at the ground hydrophone of buoy AB1 and reduced by 6 km s<sup>-1</sup>. The travel time curves are the ones calculated for the model in the lower part.

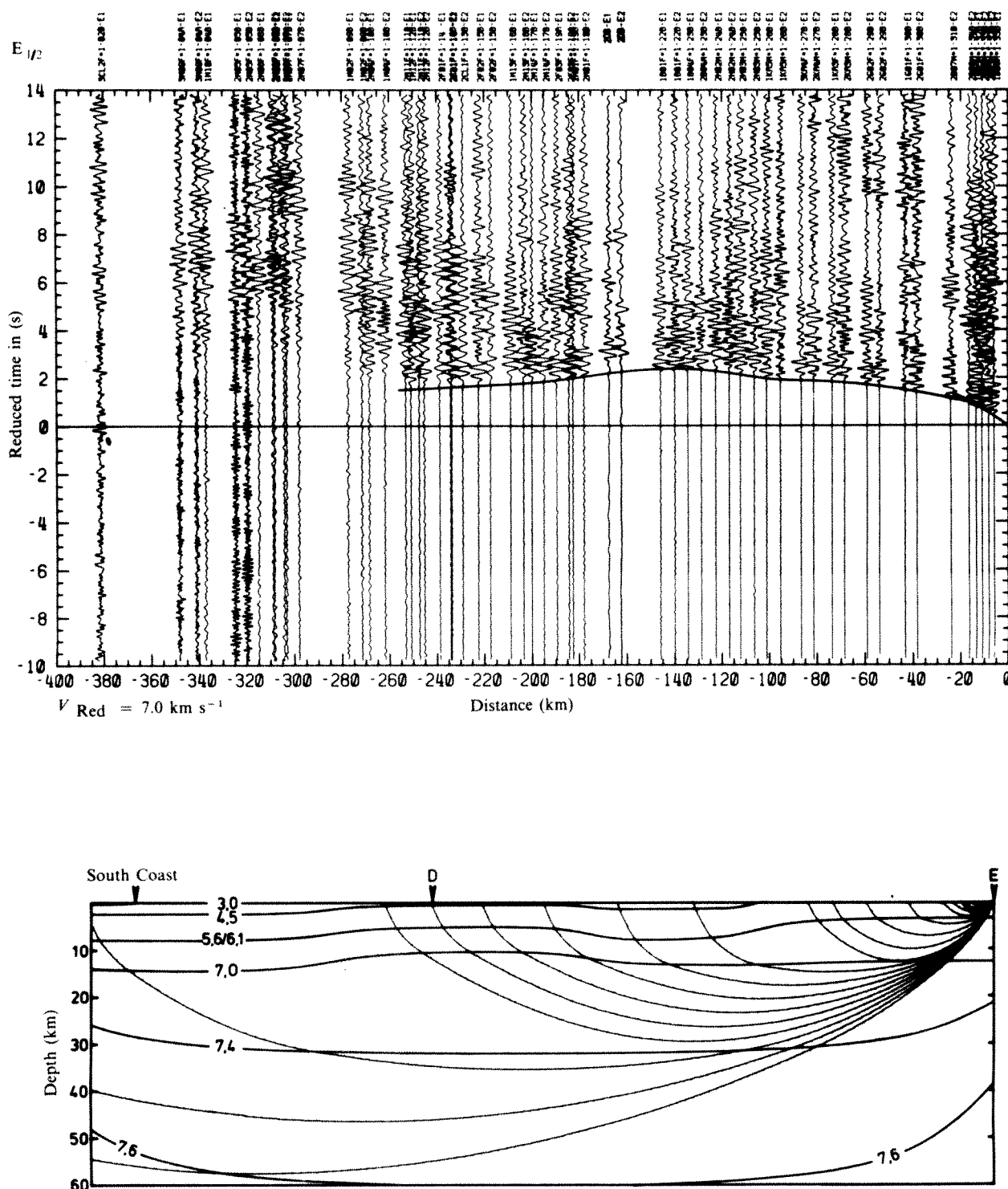


shear waves. The evaluation of both P- and S-waves, gives a normal ratio (1.76) for the P- to S-wave velocity in the uppermost 10–15 km, but a highly anomalous value (2.0) at greater depth, which may indicate a partially molten state of the anomalous mantle. This is also supported by magnetotelluric data<sup>20</sup>.

In conclusion, Iceland is not simply an uplifted part of the Mid-Atlantic Ridge, but rather a deep-seated anomaly. Yet it

looks much more like an extremely active part than an obstacle to seafloor spreading in the North Atlantic.

We thank all who made this experiment possible including many helpers from participating countries and the crew of RV Meteor. Iceland State Broadcasting Service made communication possible, Stefan Sigurdson helped to organise field work and Geophysikalisches Institut, Karlsruhe, made its computer available for digitising the MARS-66 recordings.



**Fig. 3** Record section on the main RRISP line from shotpoint E towards south-west, reduced by  $7 \text{ km s}^{-1}$ . The travel time curve is the one calculated for the model in the lower part and only few of the calculated rays are shown. The model is defined by lines of equal velocity, indicated by the numbers inserted, and by linear vertical interpolation between neighbouring isolines. The depth range below the  $7.0 \text{ km s}^{-1}$  isoline is interpreted as anomalous mantle.

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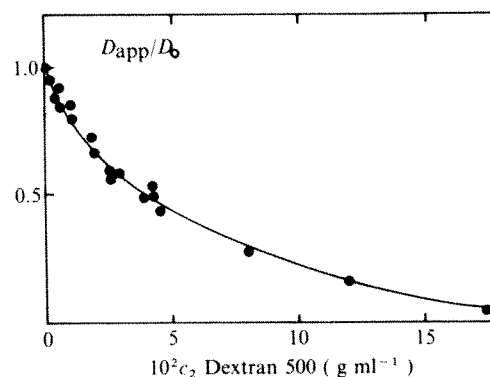
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## Transport of molecules in concentrated systems

TRANSPORT of molecules in concentrated systems is of fundamental importance in medicine, biology and technology, for example, transport within cartilaginous tissues, the release and distribution within the body of encapsulated drugs and transport of reactants in the production process of plastic materials. These systems are usually multicomponent in nature. In all these processes the transport of substances often has a regulatory role as a rate determining step. An understanding of the transport process of macromolecules in multicomponent



**Fig. 1** Reduced diffusion coefficient ( $D_{app}/D_0$ ) of bovine serum albumin (BSA) (component 1) as a function of the concentration of Dextran T 500 (component 2; molecular weight  $5 \times 10^5$ ).  $D_0$  is the diffusion coefficient of BSA in the absence of added dextran (B. N. P. and J. D. Wells, unpublished observations).

systems is therefore essential. Although problems of this type have been treated in a few theoretical<sup>1–7</sup> and experimental<sup>8–20</sup> studies, the understanding of these systems is incomplete, especially for concentrated solutions. It is known from studies of diffusion in binary systems that a number of features of the transport process appear that do not show up in more dilute systems<sup>7,10,18–20</sup>. We present here new data for macromolecular systems which show that the diffusion rate of one macromolecule can be 'paradoxically' increased considerably by adding a sufficient amount of a suitable second macromolecular component. An interpretation of this effect is advanced in terms of an extension of existing theories of concentrated solutions.

The following discussion will be restricted to one homogenous phase, ignoring, for the moment, transport across phase boundaries such as membranes. The results apply, however, to transport inside a living cell or in the region between cells or inside a membrane.

The rate of diffusion transport<sup>1,21</sup> (diffusion flow,  $J$ ) in a binary system (solute + solvent) is determined by the value of the diffusion coefficient,  $D$ , and by the gradient in solute concentration,  $c$ , according to the relationship

$$J = -D \text{ grad } c \quad (1)$$

For a given concentration gradient (in living matter a steady state situation is often prevalent) the flow rate is determined by the numerical value of  $D$ . This coefficient depends on the thermodynamic as well as the hydrodynamic properties of the system according to the expression<sup>1,7,21</sup>

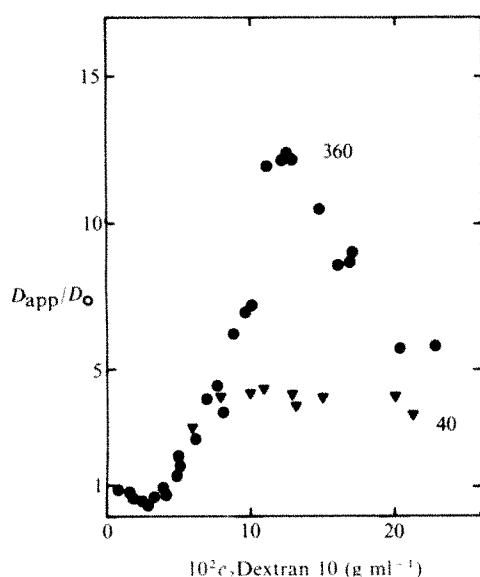
$$D(c) = \frac{k \cdot T}{f(c)} \cdot Q(c) \quad (2)$$

where  $Q(c)$  is a thermodynamic quantity, essentially the derivative of the virial expansion for the osmotic pressure, and  $f(c)$  the frictional coefficient, a hydrodynamic parameter which is a measure of the flow resistance exerted by the medium on the solute.  $k$  is the Boltzmann constant, and  $T$  absolute temperature. When concentration increases both the 'thermodynamic factor'  $Q(c)$  and the 'hydrodynamic factor'  $f(c)$  usually increase and it is the interplay between these two quantities that determines the concentration dependence of  $D$ . The numerical values of  $Q$  and  $f$  and their functional form in terms of  $c$  depend on solute-solvent system, on the size and conformational properties of the macromolecular solute as well as on temperature and pressure. For macromolecules,  $Q$  is often the dominant factor and both the diffusion coefficient and the thermodynamic driving force may change by powers of 10 when the concentration is increased from infinite dilution to, say, 10% by weight<sup>18</sup>.

In multicomponent systems the situation is more complex due to the coupling of flows and the possibility of thermodynamic

interaction between solutes<sup>22</sup>. In a ternary system (solute 1 + solute 2 + solvent), for instance, there are two independent flows,  $J_1$  and  $J_2$ , and four diffusion coefficients related through the Onsager reciprocal relationship, thus leaving three of them as independent quantities. However, if in such a ternary system the concentration of solute 1 is low and if the interest is focused on how the rate of transport of this solute is affected by successive additions of solute 2, expressions of the general form (1) and (2) can still be used<sup>24</sup> as good approximations of reality.  $D$  is then to be interpreted as an apparent diffusion coefficient,  $D_{app}$ , governing the flow of component 1.

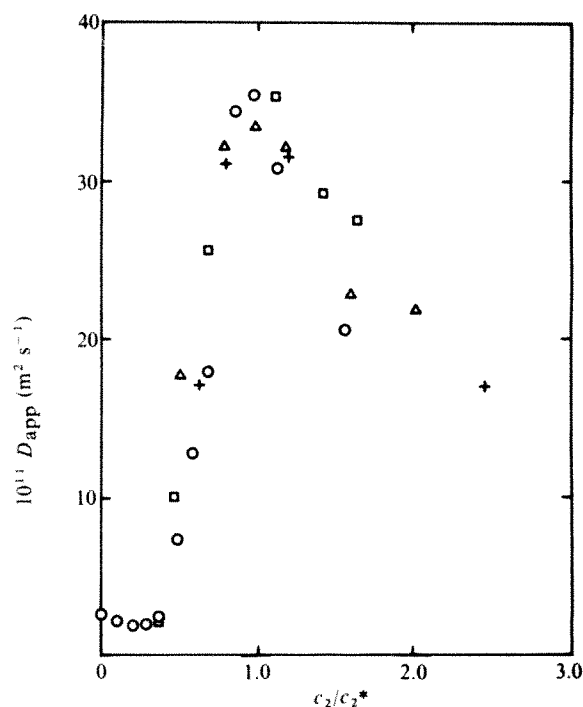
$D_{app}$  depends on both the thermodynamic and the hydrodynamic interaction between solutes. Although there is a lack of systematic studies of thermodynamic interactions, certain osmotic pressure and light scattering data<sup>23-25</sup> obtained for ternary systems indicate that in the case of two different chain polymers the interaction is strong (large excluded volume effects) and increases considerably with increasing concentration of one or both of the solutes (successively higher order



**Fig. 2** Reduced diffusion coefficient ( $D_{app}/D_0$ ) of polyvinylpyrrolidone (PVP) (component 1) as a function of the concentration of Dextran T 10 (component 2;  $MW = 10 \times 10^4$ ). ●, PVP 360 ( $\bar{M}_n = 3.2 \times 10^5$ ); ▼, PVP 40 ( $\bar{M}_n = 4 \times 10^4$ ). (B. N. P. and R. G. Kitchen, unpublished observation).

terms in the viral expansion gain importance). The frictional properties of multicomponent macromolecular systems are less known. From some sedimentation and diffusion results<sup>4,5,9,12,14,16,24,26</sup> and general knowledge about binary systems one may conclude, however, that the frictional coefficient will increase markedly on addition of the second solute, but less so for intermediate concentrations than does the thermodynamic factor (see below).

Recent experiments<sup>17</sup> on the diffusion of one macromolecule (component 1) in dilute form in a solution containing increasing amounts of a second macromolecule (component 2) up to very high concentrations have revealed the following features. If component 1 is a compact globular particle (for example, bovine serum albumin (BSA)) diffusing in a solution containing dextran (component 2), a smooth decrease in  $D_{app}$  is observed (Fig. 1). These results are in agreement with earlier work<sup>9,14</sup>. However, if both solutes are chain polymers, then a marked difference in behaviour is observed (Fig. 2). In this case, the diffusion rate of polyvinylpyrrolidone (PVP) first decreases with increasing  $c_2$ ,



**Fig. 3** Variation in  $D_{app}$  of PVP ( $\bar{M}_n = 3.2 \times 10^5$ ) (component 1) as a function of  $c_2/c_2^*$  for various dextrans (component 2) of different molecular weight. ○, Dextran T 10 ( $MW = 1.0 \times 10^4$ ); □, Dextran T 20 ( $MW = 2.0 \times 10^4$ ); △, Dextran T 70 ( $MW = 7.0 \times 10^4$ ); +, Dextran T 150 ( $MW = 15.0 \times 10^4$ ).  $c_2^*$  is calculated using a modified version<sup>29</sup> of a relationship as given by Simha and Zakin<sup>28</sup>. (B. N. P. and G. Checkly, unpublished observations).

then increases markedly;  $D_{app}$  can reach very high values sometimes of the order of 45 times the value in the absence of component 2. Then  $D_{app}$  reaches a maximum and decreases at still higher values of  $c_2$ . These results point out the regulatory influence of the second solute on the diffusion rate of the first.

These results seem to be in accord with the general view of diffusion in multicomponent macromolecular systems as discussed above, with the following qualitative explanation. The diffusion rate is governed by an interplay between 'thermodynamic' and 'hydrodynamic' factors. Component 1 is present in high dilution and, up to a certain but fairly low value of  $c_2$ , the addition of component 2 mainly affects frictional properties and the diffusion rate of component 1 is lowered. When  $c_2$  has been raised sufficiently the thermodynamic interactions between the different macromolecules will, in the case of the linear polymers, rapidly become very strong due to large excluded volume effects and will dominate over the increase in friction. This explains the very steep increase in the  $D_{app}$  curve against  $c_2$ . Continued addition of component 2 finally gives a situation where close packing of the encompassed-volume-spheres of the solute molecules occurs. For dense hexagonal close packing, it has been suggested that this occurs at a concentration ( $c_2^0$ ) given by  $c_2^0 = 1.08/[\eta]$  where  $[\eta]$  is the intrinsic viscosity of solute 2 (ref. 28). At this concentration, there will be a drastic increase in the 'friction' which eventually will dominate over the thermodynamic interactions.

If the molecular weight and hence molecular domain of component 2 in the system studied in Fig. 2 is increased, then the resultant  $D_{app}$  against  $c_2$  curve is displaced to lower concentrations. However, if a normalised concentration scale is used,  $c_2/c_2^*$ , where  $c_2^*$  differs from  $c_2^0$  only by a small factor that allows for the compression of solute 2 (B. N. P. and A. G. Ogston, in preparation), then the curves  $D_{app}$  against  $c_2/c_2^*$  become superimposable (Fig. 3). It appears that the reduced scale defines approximately corresponding physical states for the



different molecular weight species. It is pertinent that the maximum value of  $D_{app}$  occurs at the point of incipient overlap of the molecular domains of component 2 (that is  $c_2/c_2^* = 1$ ).

That the molecular conformation of the diffusing species is critical in determining the diffusion features has been clearly demonstrated by study of the simultaneous diffusion of PVP and BSA in a concentrated solution of dextran. Whereas the diffusion transport of the chain polymer was enhanced by a factor of 12, that of albumin was reduced by a factor of 2 (unpublished observation).

This small note precludes a detailed theoretical examination of multicomponent diffusion and thermodynamic interaction but a number of features predicted by theory have been observed in the system discussed above. These include the marked dependence of  $D_{app}$  on: (1) molecular weight of component 1 (Fig. 2); (2) the concentration of both polymeric components; and (3) time.

Our observations may be relevant for understanding the rate and direction of diffusion in extracellular compartments containing high concentrations of, for example, polysaccharides or mucins. However, they may also be important for intracellular processes such as axonal flow.

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## Ear ossicle of *Australopithecus robustus*

WE report here the discovery of the first ear ossicle, an incus, of a Plio–Pleistocene hominid. It is substantially different from that of modern man, and the dissimilarity exceeds that between the ear bones of *Homo sapiens* and of the African apes. The new incus is of interest particularly in view of the unique advantages that ear ossicles have for taxonomic and phylogenetic studies. (The only other fossil hominid ear ossicles are from Qafzeh<sup>1</sup> and are indistinguishable from those of modern man.)

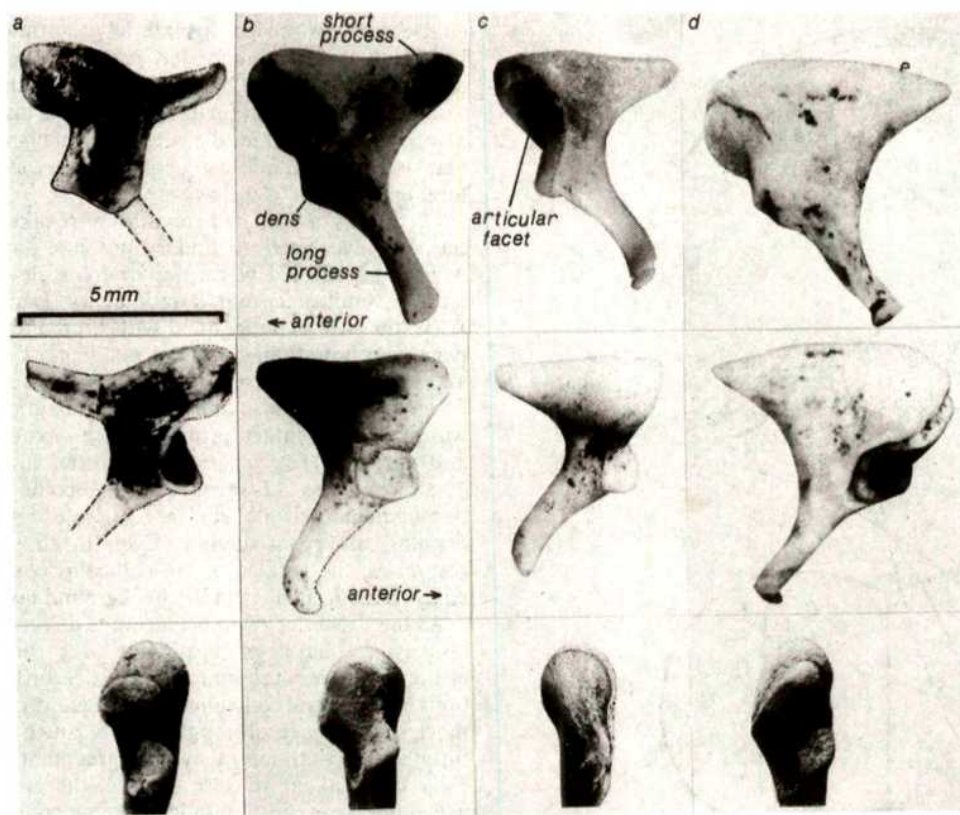
The right incus was discovered in the *Australopithecus robustus* specimen SK 848, a temporal bone from Swartkrans (a distinct genus, *Paranthropus*, is recognised by one of us, R.J.C.). The incus was found anatomically *in situ*—in the epitympanic recess—and is complete except for the long process (Fig. 1). The near-perfect state of preservation permits detailed examination of the bone's morphology, including the articular surface.

The body of the Swartkrans incus is inflated and bulb shaped. A deep indentation at the centre of the articular surface emphasises the protrusion of the oversized dens, thus providing the body with a somewhat more symmetrical appearance than in man, gorilla and chimpanzee. The short (posterior) process is cylindrical and of small diameter. The area of its attachment to the body is narrow compared with the corresponding area in man and the African apes, where the process is flat and broadens anteriorly to meet the body. In *A. robustus* the small diameter of the process results in a concave superior contour and the elevation of the body above the process.

Comparison of the articular surfaces also reveals profound differences. The synovial incudomalleolar joint in man is usually described as a saddle-shaped biaxial joint, and in this respect it resembles the chimpanzee and the gorilla. The saddle shape enables the superior part of the articular facet to be exposed in medial view (Fig. 1*b, c, d*, top) and the inferior part (on the dens) to be exposed in lateral view (Fig. 1*b, c, d*, middle). This twisting of the surface from the medial to the lateral sides of the bone is not found in *A. robustus*, where a view of the medial side does not include the articular surface (Fig. 1*a*, top). The two parts of the articular surface (superior and inferior) face each other, forming a more uniaxial joint shaped like the semilunar notch of the ulna. Only in *A. robustus* does the superior part of the articular surface occupy such a small portion of the bone's body (Fig. 1*a*, bottom).

Although the incus is tiny, its importance should not be overlooked. Furthermore, this is one of the most complete and undistorted bones of *A. robustus* yet discovered. The study of its morphology with respect to taxonomy and phylogeny is aided by additional advantages unique to this bone. It is fully formed at birth, and thus later changes are minimal. It has no muscles anchored on it and so exhibits none of the morphological effects often associated with muscular attachment. As Hershkovitz (ref. 2, p. 176) states in reference to the ear ossicles, "Their growth patterns, size, shape, position, and composition are not significantly influenced or modified by movements or stresses generated by the growth or remodeling of unrelated neighboring bones. In effect, ontogeny of the ossicle is virtually a complete and undisturbed expression of the basic controlling genetic factors. Importance of ear bone morphology and ontogeny in phylogenetic reconstructions cannot be underestimated." This is more true of the incus than the malleus<sup>3–5</sup>.

Specialisations of the ear are usually found in structures other than the ossicles, such as the pinna and the external auditory meatus, the tympanic bulla, the fenestra ovalis and the ligaments supporting the ossicles; specialisations are also exhibited by certain anatomical relationships, such as the proportion between the areas of the tympanic membrane and the fenestra ovalis<sup>3–5</sup>. Many of the modifications associated with the ear ossicles themselves are related to the position and orientation of



**Fig. 1** Comparison of the right incus of *A. robustus* (a) with that of modern man (b), chimpanzee (c) and gorilla (d). Top row, medial view of the bone; middle row, lateral view; bottom row, view of the articular surface. All bones are on the same scale and orientation.

these bones in the tympanic cavity and to the elongation of their processes<sup>3-5</sup>. Furthermore, the malleus—the most external ossicle in the chain—tends to be more specialised than the incus<sup>2-5</sup>. Only in cases of extreme modification of the ear, as in bats, moles and whales, is the morphology of the incus substantially affected<sup>3-5</sup>. It is interesting that the acute angulation of the articular facet's surface, which distinguishes the *A. robustus* incus from that of the African apes and man, is also characteristic of the rodent *Dipodomys merriami*, whose ear is specialised to an unusually low frequency range<sup>3</sup>. Furthermore, the extremely delicate short process in *A. robustus* (the site of attachment of the posterior ligament connecting the incus to the petrous bone) may indicate that, as in *D. merriami*, the ossicles were loosely suspended in the tympanic cavity<sup>3</sup>.

The solid appearance of the SK 848 incus and of its articular surface suggests that its shape is not the result of some pathological process. Its unusual morphology is far beyond the range of normal variation characteristic of the incudes of modern man and the great apes<sup>4</sup>. This is also supported by the observations made by one of us (Y.R.) on tens of *H. sapiens* bones from different populations and on five gorilla and 10 chimpanzee bones.

Although the specific phylogenetic and taxonomic implications of our findings are unclear because of the lack of a comparative fossil incus sample, this bone can provide at least a notion of how great a phylogenetic deviation is represented by the robust australopithecines.

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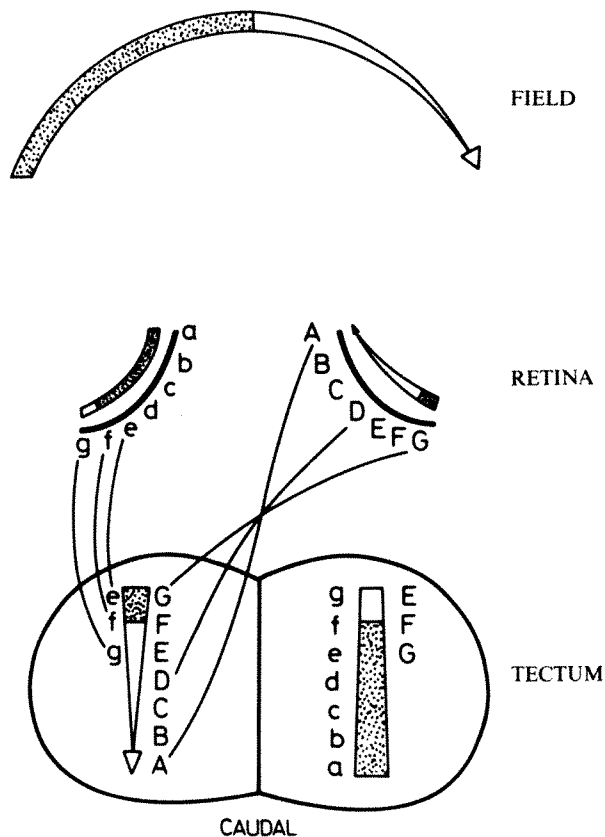
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## Changes in the uncrossed retinotectal projection after removal of the other eye at birth

THE connections between the eyes and the brain are so arranged as to give an orderly representation in the brain, of the visual field. The mechanisms involved in this ordering have been extensively studied, particularly in the retinotectal pathway of submammalian vertebrates<sup>1-3</sup>. In these species the optic nerves decussate almost completely, retinal ganglion cells in one eye connecting only with the opposite tectum. In mammals, however, there is partial decussation at the optic chiasma, so that the superior colliculus receives optic fibres from both eyes. Binocular representation poses a problem for the ordering of retinotectal projections. If one point on the tectum is to correspond to only one point in visual space, seen through both eyes, the mapping rules onto the tectum must have opposing polarities in the two eyes, along the nasotemporal axis. This is shown in Fig. 1: a rostral movement in the colliculus corresponds





**Fig. 1** A schematic diagram showing the representation of the visual field on the retina and on the tectum of a mammal such as the hamster. The horizontal meridian of the visual field is depicted as an arrow and is shown projecting onto the two tecta so that the central field is found rostrally and the peripheral field caudally. There are connections from the temporal retina of both eyes to the rostral portion of each tectum such that one point on the tectum corresponds to only one position in the central visual field, seen through both eyes. It is apparent that the ordering of the projection shows opposing nasotemporal polarities in the ipsilateral and contralateral retinas. In left tectum the sequence G F E from the contralateral eye is matched by e f g in the ipsilateral eye; in the right tectum g f e is matched by E F G.

to a temporal movement on the contralateral retina but a nasal movement on the ipsilateral retina. Little is known about the factors determining the topography of the direct ipsilateral projection to the mammalian superior colliculus. Anatomical studies in rodents have shown that neonatal removal of one eye induces an increased projection from the remaining eye to the ipsilateral colliculus<sup>4,5</sup>. These aberrant uncrossed optic fibres are derived from all regions of the retina and apparently have a distribution appropriate for a normal contralateral projection, that is, the nasotemporal retinal axis is represented caudorostrally<sup>6</sup>. The only published physiological study investigating the topography (in enucleated rats) confirmed the anatomy as regards the nasotemporal axis but, surprisingly, found the dorsoventral axis reversed over most of the colliculus ipsilateral to the remaining eye<sup>7</sup>. Here I present results on the enucleated hamster showing that the colliculus ipsilateral to the remaining eye contains a double representation of the visual field displaying mirror-image polarity along the nasotemporal axis.

I have examined electrophysiologically the retinal representation in the superior colliculi of hamsters, *Mesocricetus auratus*, which had one eye removed at birth. I used both normally pigmented and albino strains in an attempt to distinguish the contributions to the retinotectal map of fibres which would normally have gone ipsilaterally, and of fibres induced by

enucleation, to project ipsilaterally. Normal albino mammals have only a small uncrossed retinal projection<sup>8</sup> and I have confirmed this for the hamster using both anatomical and physiological techniques (in preparation). This difference in the extent of the ipsilateral projection in pigmented and albino hamsters makes it interesting to compare the effects of contralateral enucleation in the two strains.

On the day of birth hamster pups were taken from the nest and the right eye removed under fluothane anaesthesia. Animals over 3 months old were prepared for electrophysiology in a manner similar to that described by Tiao and Blakemore<sup>9</sup>. Anaesthesia was maintained with urethane-chloralose (25 mg per kg per h urethane; 0.16 mg per kg per h chloralose) and the animals were paralysed with gallamine triethiodide (Flaxedil, 35 mg per kg per h). After removing the dura the visual cortex was usually left intact, protected by a mixture of liquid-paraffin and vaselife. The electrode (tungsten in glass<sup>10</sup>, tip length 12–18  $\mu$ m) was advanced into the colliculus approximately perpendicular to its surface; microlesions were made for histological reconstruction. Even in the absence of visual responses, the surface of the colliculus could be identified by characteristic changes in the background noise.

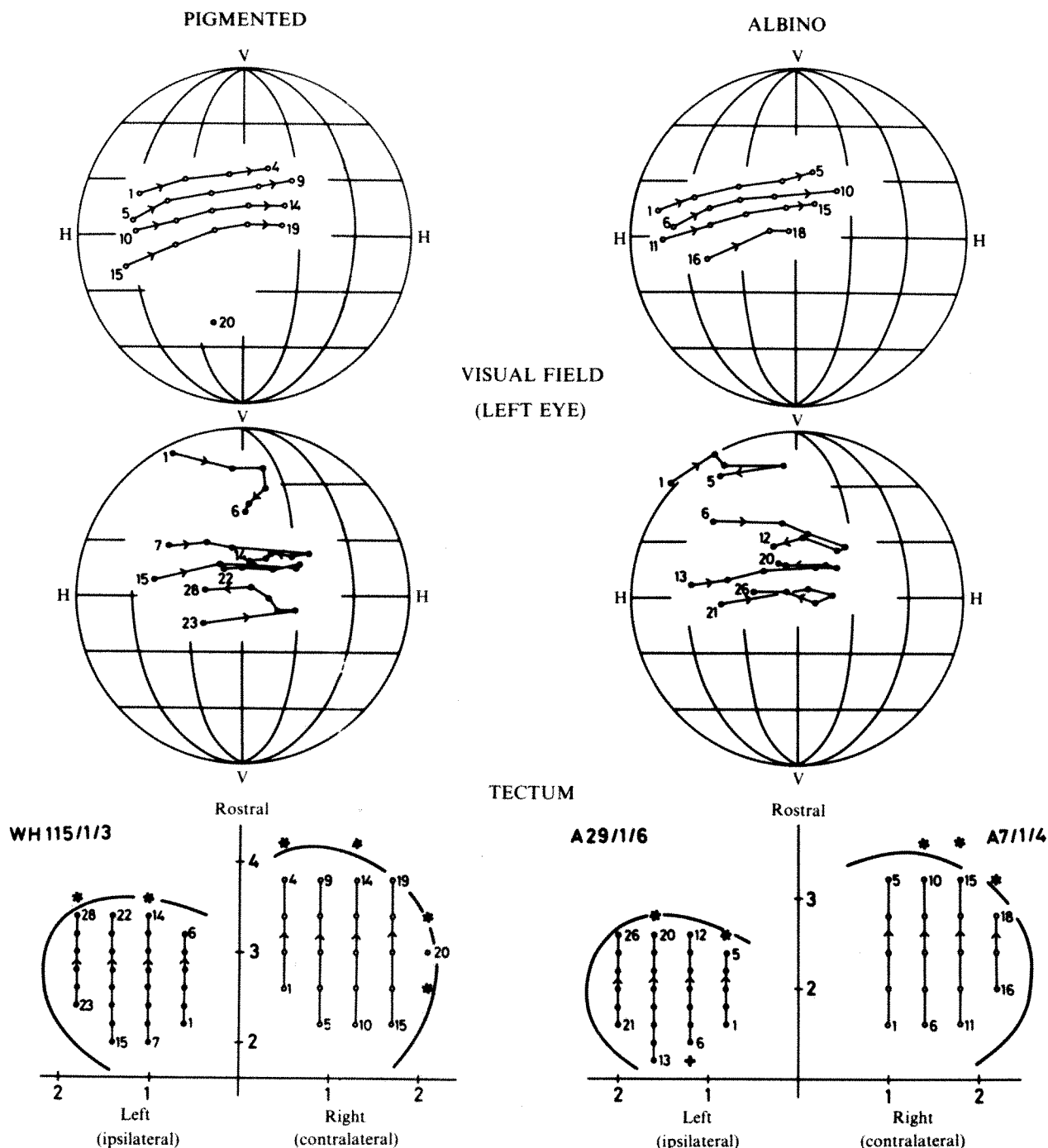
As the existence of collicular units driven through the ipsilateral eye<sup>11–13</sup> has been questioned<sup>14</sup>, recordings were first made in normal pigmented animals. Cells recorded during penetrations in the rostral colliculus were frequently responsive through the ipsilateral eye although usually much more weakly than through the contralateral eye, in agreement with the findings of Tiao and Blakemore<sup>11</sup>. In general, the part of the tectum in which there were ipsilaterally evoked responses agreed with the distribution of uncrossed optic terminals defined by anatomical methods (in preparation). In addition, receptive fields in the two eyes always shifted together in visual space as the electrode was moved across the colliculus. Consequently, the situation shown in Fig. 1 does represent that of the normal hamster.

Recordings were made in 12 enucleated animals (six albino and six normally pigmented): 299 penetrations into the mid-brain ipsilateral to the remaining eye and 50 in the opposite side (two animals). Multi-unit activity was recorded as soon as the electrode entered the colliculus and the receptive field was plotted by moving spots of light on a hemispherical screen in front of the animal. Receptive fields were generally 10–20° in diameter, occasionally much larger, but no double receptive fields were seen<sup>7</sup>. Compared with the visual responses from the ipsilateral eye in the normal animal, those in the enucleated animal were much brisker and were recorded over a far greater extent of tectum.

Figure 2 shows typical collicular maps of the visual field for animals enucleated at birth and recorded when adult. A comparison of the uncrossed and crossed projections in one animal (Fig. 2, WH 115/1/3) shows that there is a reversal within the retinotectal topography of the uncrossed projection. Whereas a caudorostral movement in the colliculus contralateral to the remaining eye gives a normal monotonic progression from temporal to nasal visual fields, such a movement in the ipsilateral colliculus corresponds initially to a temporonasal field progression, which then reverses to move from nasal to temporal fields. The polarity of the retinotopic map in the rostral tectum is appropriate for a normal ipsilateral projection whereas that in the caudal tectum is appropriate for a projection to the contralateral side.

This pattern of ipsilateral retinotectal topography following removal of one eye at birth, was seen in all 10 animals in which the mapping was sufficiently extensive. There is no obvious difference between pigmented and albino animals (Fig. 2). The retina maps continuously onto the tectum but with two polarities; consequently, parts of the retina are represented in two places on the tectum. It is only the central part of the visual field (temporal retina) that is mapped twice. As in the normal animal, the rostral colliculus represents only the central (normally binocular) visual field. The aberrant projection to the caudal colliculus includes both central and peripheral visual field and is





**Fig. 2** The mapping of the visual field, seen through the left eye, onto the superior colliculi, is shown for the pigmented hamster (left) and the albino (right). The right eye of each hamster had been removed on the day of birth. Filled circles indicate the locations of penetrations into the left colliculus (ipsilateral to the remaining eye) and also the position of the corresponding receptive fields in visual space; contralateral penetrations and the matching receptive fields are depicted by open circles. The electrode, which was angled 35° forward from the vertical in the parasagittal plane, was moved in a regular matrix of penetrations over a horizontal stereotaxic plane through lambda. The axes (in mm) at the bottom of the figure plot the intersections of the penetrations with this plane, the origin being the lambda. The points representing each parasagittal line of penetrations are joined and the progression from caudal to rostral tectum is indicated by the arrowheads. Asterisks denote penetrations falling outside the colliculus and the cross refers to one penetration within the colliculus on which no visual response could be elicited. Above the collicular representations are the visual field maps. In each case, the upper map (open circles) is derived from penetrations in the contralateral (right) colliculus and the lower (filled circles) from ipsilateral penetrations. The visual field coordinate system is that used by Tiao and Blakemore<sup>11</sup>, using axis-vertical coordinates for the hemisphere whose anterior pole lies directly in front of the animal. The meridians and parallels are shown at 30° intervals; H-H and V-V are the horizontal and vertical meridians. To compensate for divergence of the eyes under relaxant, a small correction was applied to the positions of the receptive fields, assuming that the normal projection of the optic disk (monitored with an ophthalmoscope) lies 54° from the vertical meridian and 25° above the horizontal meridian<sup>11</sup>.

consequently compressed compared with the normal crossed projection.

Thus, after removal of one eye at birth, the uncrossed fibres exhibit retinotectal mapping polarities appropriate for both contralateral and ipsilateral projections in the normal animal. This implies that the mechanisms involved in determining these polarities are independent of whether or not decussation occurs and that the map in the rostral colliculus, appropriate for a normal ipsilateral projection, does not require the presence of an intact projection from the opposite eye. A further surprising feature is the segregation of the two maps in the ipsilateral tectum. The aberrant input showing mapping rules appropriate to a contralateral projection might have been expected to fill the whole colliculus, with the two maps superimposed in the rostral half as they are in the normal animal (where they come from different eyes).

This segregation could arise if the aberrant axons, induced to project ipsilaterally by enucleation of the other eye but exhibiting contralateral mapping rules, arrive at the tectum much later than would a normal crossed projection and find themselves excluded from the rostral tectum because of proliferation of the normal ipsilateral termination in that region. However, this argument might predict that the area of rostral tectum occupied by a normal ipsilateral map should still be very small in the enucleated albino; surprisingly, it is not (Fig. 2). Thus, in albinos at least, some of the fibres that are induced to project ipsilaterally, nevertheless, behave appropriately for a normal ipsilateral projection; perhaps these are the fibres that the albino mutation would normally cause to pass contralaterally rather than ipsilaterally<sup>15-17</sup>.

An important question is whether the physiologically determined retinotectal map actually reflects the pattern of direct retinal ganglion cell input<sup>18,19</sup>. In this context further anatomical work is necessary to ascertain whether the apparent discrepancy between the maps found in this study and those determined anatomically in the rat<sup>6</sup> is real. A further complication is the existence in mammals of a projection from the visual cortex to the superior colliculus<sup>20,21</sup>, the removal of which, in the cat, decreases ipsilateral driving of tectal neurones<sup>22</sup>. Cunningham and Speas<sup>7</sup> removed the cortex before recording, but it is unlikely that this explains the disparity between their results and mine, as I found that removal of the visual cortex, in two animals, had no significant effect on the topography of the ipsilateral projection mapped before and after the lesion. The topography was also unaffected by subsequent removal of the colliculus contralateral to the remaining eye.

These findings imply that the normal direct projection from the eye to the ipsilateral tectum (occurring to a significant extent only in mammals) has an inherent mapping specificity which is independent of input from the other eye. The observation that two maps of different polarity, arising from one eye, can co-exist within a single tectum may mean that a single area of retina contains two populations of ganglion cells exhibiting opposed polarity labels. Alternatively, it could be that the caudal and rostral halves of the tectum provide different mapping cues for fibres entering from the ipsilateral eye. A further complication arises if uncrossed fibres in both normal and enucleated animals are branches of crossed fibres, as has been suggested for the rat<sup>23,24</sup>. In that case, the two branches of a single retinal ganglion cell would have to display different mapping rules according to their laterality. Further work is required to provide fundamental information about the formation of visual maps in the mammalian brain.

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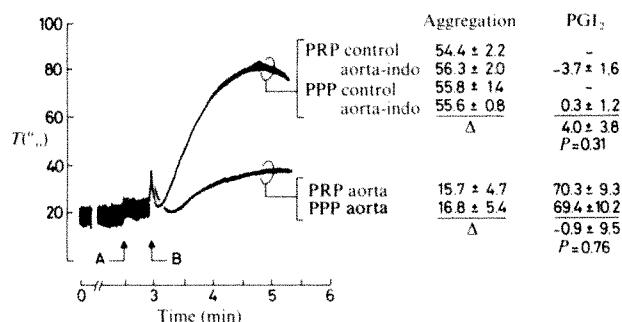
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## Blood platelets do not provide endoperoxides for vascular prostacyclin production

PROSTACYCLIN (PGI<sub>2</sub>), the most active natural inhibitor of blood platelet aggregation described so far, is synthesised from the cyclic endoperoxide, PGH<sub>2</sub>, derived from endogenous arachidonic acid by the cyclo-oxygenase enzyme system. Exogenous endoperoxides are also readily converted into PGI<sub>2</sub> (refs 1, 2). Indomethacin inhibits the cyclo-oxygenase activity and thereby prevents PGH<sub>2</sub> formation. Because of this lack of precursor, endogenous PGI<sub>2</sub> production of indomethacin-treated tissue does not occur. However, when vascular tissue, pretreated with indomethacin, is incubated in platelet-rich plasma (PRP), its PGI<sub>2</sub> production is restored. On the basis of this observation it has been suggested that activated blood platelets can be a source of exogenous endoperoxides, stimulating the vascular prostacyclin formation and, consequently, limiting the growth of a platelet thrombus<sup>3</sup>. We now report results indicating that it is highly unlikely that blood platelets are able to promote vascular prostacyclin formation by supplying cyclic endoperoxides.

Male Wistar rats, specific pathogen-free, mean body weight about 300 g, were bled under ether anaesthesia by puncturing the abdominal aorta. The blood was collected in sodium citrate (3.8 w/v % 1 part + 9 parts of blood) and PRP and platelet-poor plasma (PPP) were prepared by differential centrifugation. Aortas were removed, cleaned from adjacent tissue, opened longitudinally and kept in Krebs-Henseleit buffer with or without indomethacin (2 µg ml<sup>-1</sup>) for at least 60 min at 0°C. A small piece of tissue (diameter 3 mm, dry weight ~100 µg) was punched out of the aorta and incubated immediately in 200 µl of either PPP or PRP in a gently shaken plastic vial for 3 min at room temperature. Measurement of the prostacyclin content of the incubate was based on the inhibiting effect of the incubate on ADP-induced aggregation of blood platelets. Incubation of normal aortic tissue resulted in the production of an aggregation-inhibiting principle with typical prostacyclin characteristics<sup>4</sup>. No significant difference in PGI<sub>2</sub> production was observed on incubation with either PPP or PRP (Fig. 1).

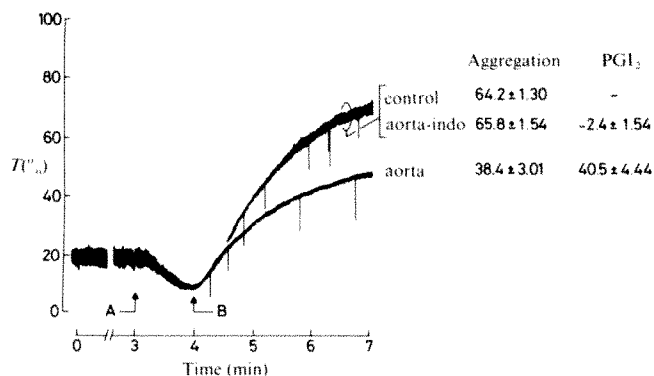
Indomethacin-treated tissue did not produce prostacyclin, irrespective of whether it had been incubated in PPP or PRP (Fig. 1). Similar results were obtained with longer incubation times (up to 12 min) and/or using human PPP and PRP as incubation media. Prostacyclin formation of normal aortas was considerably stimulated and that of indomethacin-treated aortas completely restored when PGH<sub>2</sub> was added to the



**Fig. 1** Prostacyclin production of rat aortic tissue, some treated with indomethacin (aorta-into), on incubation (3 min) in platelet-poor rat plasma (PPP, platelet count  $8-10 \times 10^3 \mu\text{l}^{-1}$ ) or platelet-rich rat plasma (PRP, platelet count  $1.7 \times 10^6 \mu\text{l}^{-1}$ ) at room temperature. The incubate (see text) was centrifuged (45 s; Beckman 152 microfuge) and 1 min after termination of incubation 50  $\mu\text{l}$  was added (A) to an aggregometer cuvette (Born/Michel) containing 0.75 ml saline (0.85 w/v % NaCl, 7 parts +  $\text{CaCl}_2 4 \times 10^{-3} \text{ mol l}^{-1}$ , 1 part) and 0.1 ml rat PRP, which had been preincubated (37 °C) for 2.5 min. Thirty seconds after incubate addition 0.1 ml ADP was added (B) to a final concentration of  $2.5 \times 10^{-7} \text{ mol l}^{-1}$ . Aggregation is indicated by the % change in light transmission ( $\Delta T$ ). Prostacyclin content of the incubates was calculated from the difference in aggregation in the presence of aorta-containing incubates (b) compared with aorta-free incubates (a) and expressed as % inhibition:  $\text{Inh. \%} = (a - b/a)100\%$ . Mean values  $\pm$  s.e.m. of seven determinations.

incubation medium ( $1 \mu\text{g ml}^{-1}$  final concentration), thus showing the responsiveness of the tissue to exogenous precursor in the present experimental conditions. Therefore, it can be concluded that PRP, gently shaken at room temperature for 3 min, does not supply the vascular wall with endoperoxides for prostacyclin synthesis.

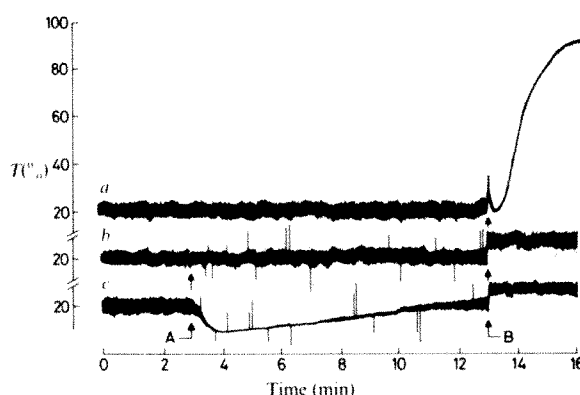
Because this PRP treatment might not have activated the platelets sufficiently to initiate arachidonate peroxidation, in the next series of experiments platelet endoperoxide production



**Fig. 2** Prostacyclin production of rat aortic tissue which had (aorta-into) or had not (aorta) been preincubated ( $\geq 60$  min) in indomethacin-containing Krebs-Henseleit buffer ( $2 \mu\text{g ml}^{-1}$ ). Aggregation of rat PRP (platelet count  $1.7 \times 10^6 \mu\text{l}^{-1}$ ) was triggered (A) by collagen ( $5.6 \times 10^{-6} \text{ g protein ml}^{-1}$  final concentration). At the beginning of aggregation (delay time  $\sim 60$  s) aortic tissue was added (B). Aggregation was quantified as the % change in transmission 3 min after adding the tissue ( $\Delta T$ ). Prostacyclin production was determined by measuring the difference in collagen-induced aggregation after tissue addition (b) compared with control aggregations without added tissue (a) and expressed as % inhibition:  $\text{Inh. \%} = (a - b/a)100\%$ . Mean values  $\pm$  s.e.m. of 16 determinations.

was triggered by collagen. PGI<sub>2</sub> formation by normal and indomethacin-treated aortic tissue was assessed on incubation in PRP during aggregation induced by a collagen dose that caused platelets to produce endoperoxides (measured as malondialdehyde) and to release part of their granule content<sup>5</sup>. The results (Fig. 2) show that indomethacin-treated aortic tissue is unable to produce prostacyclin even if it is incubated in a suspension of collagen-activated blood platelets.

We then investigated whether the prostacyclin production of vascular tissue not treated with indomethacin could be influenced by incubation in PRP producing different amounts of endoperoxides. For this, we used platelets and aortas of normal rats and animals deficient in essential fatty acids (EFA). Platelets of these latter animals, when triggered by collagen, aggregate less than normal platelets<sup>6,7</sup> and produce only small amounts of endoperoxides and thromboxanes<sup>5,7,8</sup>. Their vascular prostacyclin production is lower than that in normal animals<sup>4</sup>, but thromboxane<sup>7</sup> and prostacyclin synthetase<sup>4</sup> are present in normal amounts.



**Fig. 3** Effect of normal and indomethacin-treated aortic tissue on the % light transmission ( $T$ ) of PRP and ADP-induced aggregation in PRP. A PRP dilution (for details see legend to Fig. 1) was stirred (1,000 r.p.m.) in an aggregometer at 37 °C. After 3 min (A) a piece of aorta was added. Ten minutes later (B) the tissue was removed and ADP added to trigger aggregation. a, No aorta. Aggregation indicates response of platelets to ADP. b, Normal aorta. No platelet shape change and aggregation after either tissue addition or ADP administration. In later experiments this was shown to be the result of prostacyclin formation by the added tissue. c, Indomethacin-treated aorta. The immediate decrease in transmission and the diminishing oscillations indicate activation of the platelets by the vascular tissue. After some time the tracing tended to return to normal, indicating de-activation of the platelets. Together with the absence of aggregation on ADP addition, this suggests progressively increasing prostacyclin formation, which was confirmed in later experiments (not shown).

If platelet endoperoxides stimulate vascular prostacyclin formation, one would expect more PGI<sub>2</sub> to be formed on incubation of vascular tissue with collagen-activated normal than EFA-deficient PRP. However, Table 1 shows that neither normal nor EFA-deficient aortic tissue produce more PGI<sub>2</sub> on incubation with collagen-activated normal PRP than with collagen-activated EFA-deficient PRP.

Because normal as well as EFA-deficient vascular tissue can easily be stimulated to produce more PGI<sub>2</sub> merely by adding exogenous PGH<sub>2</sub> to the incubation medium, it is highly unlikely that activated blood platelets contribute to vascular prostacyclin synthesis by providing cyclic endoperoxides. As shown by Bunting *et al.*<sup>3</sup> and confirmed by others<sup>9,10</sup>, including ourselves (Fig. 3), indomethacin-treated tissue prevents aggregation when incubated in PRP. The mechanism for this is unknown, but we



**Table 1** Prostacyclin production of rat aortic tissue on incubation with collagen-aggregated PRP of normal or EFA-deficient rats

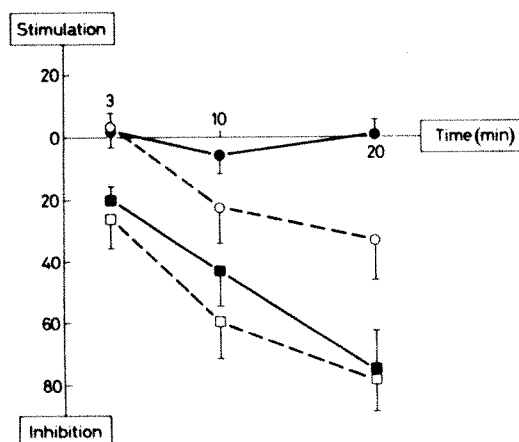
Aorta	PRP	Prostacyclin (ng PGE <sub>1</sub> per min)	Difference
Control	Control	9.1 ± 1.32	
Control	EFA-deficient	7.8 ± 0.91	$P > 0.10$
EFA-deficient	Control	1.3 ± 0.58	$P < 0.001$
EFA-deficient	EFA-deficient	1.4 ± 0.41	$P > 0.10$

For technical details see the legend to Fig. 2. As the collagen-induced aggregation is depressed in EFA deficiency<sup>6,7</sup>, effects were calibrated against the inhibitory effect of prostaglandin E<sub>1</sub> (PGE<sub>1</sub>). Dose-response curves of PGE<sub>1</sub> were made in both normal and EFA-deficient PRP. Prostacyclin production is given in PGE<sub>1</sub> equivalents (ng min<sup>-1</sup>), mean ± s.e.m. of 11 determinations. Results were statistically evaluated using the Kruskal-Wallis analysis of variance on ranked observations<sup>11</sup>.

believe that it is due to removal of indomethacin from the cyclo-oxygenase enzyme system, which is consequently reactivated during incubation. This was confirmed by the following experiment.

A piece of indomethacin-treated rat aorta was added to a stirred rat PRP dilution and the change in transmission recorded continuously. When after 10 min the tissue was removed and ADP (0.1 ml,  $2.5 \times 10^{-6}$  mol l<sup>-1</sup>) added to the PRP, no aggregation was observed (Fig. 3). Aggregation did occur, however, when the platelet mixture was stirred without added aorta (Fig. 3), confirming Bunting's observation that indomethacin-treated vascular tissue produces PGI<sub>2</sub>-like activity when incubated in stirred PRP.

The removed tissue was quickly rinsed in Krebs-Henseleit buffer, incubated in 200 µl Tris buffer (0.02 mol l<sup>-1</sup>, pH 7.2) at



**Fig. 4** Endogenous prostacyclin production (% inhibition of ADP-induced aggregation) of indomethacin-treated aortic tissue of rats after preincubation. Pieces of vascular tissue were added to an aggregometer cuvette containing 0.8 ml saline + CaCl<sub>2</sub> (see Fig. 1) and 0.1 ml PRP, PPP or Krebs-Henseleit buffer. This mixture had been preincubated for 3 min at 37°C, while stirred at 1,000 r.p.m. After 10 min the tissue was removed, rinsed in Krebs-Henseleit buffer and incubated in 200 µl Tris buffer (0.02 mol l<sup>-1</sup>, pH 7.2) at room temperature in a gently shaken vial. After 3, 10 and 20 min 50-µl portions of the incubate were assessed for their PGI<sub>2</sub>-like activity on the basis of their inhibiting effect on ADP-induced aggregation (see Fig. 1). Results are means ± s.e.m. of seven determinations. First and second media were, respectively: ■, PRP, Tris; □, PPP, Tris; ○, Krebs-Henseleit, Tris; ●, Krebs-Henseleit + indomethacin (2 µg ml<sup>-1</sup> final concentration), Tris + indomethacin (2 µg ml<sup>-1</sup>).

room temperature, and endogenous PGI<sub>2</sub> production assessed in 50-µl portions of the incubate taken after 3, 10 and 20 min of incubation. If the cyclo-oxygenase was still blocked by indomethacin, no prostacyclin production would be expected. However, substantial amounts of endogenous PGI<sub>2</sub>-like activity were detected in the incubate (Fig. 4). This is not due to 'late' conversion of stored, platelet-derived PGH<sub>2</sub>, as essentially similar results were obtained on 10 min incubation of indomethacin-treated tissue in a stirred PPP dilution or in a dilution of Krebs-Henseleit buffer (Fig. 4). Endogenous PGI<sub>2</sub> production could be prevented only when the first as well as the second incubation was carried out in a saline milieu, containing indomethacin (Fig. 4).

Indomethacin added to PRP or PPP (2 µg ml<sup>-1</sup>) did not prevent reactivation of vascular cyclo-oxygenase (not shown), most probably because the added indomethacin is bound to plasma proteins, resulting in too low a 'free' indomethacin concentration. There was no appreciable difference between the use of PRP or PPP during the first incubation on subsequent endogenous PGI<sub>2</sub> production by the tissue. This indicates that platelets do not have a special effect on reactivation of vessel wall cyclo-oxygenase. In this respect, plasma seems superior to buffer, probably because the strong affinity of indomethacin for plasma proteins facilitates its removal from vascular cyclo-oxygenase.

In our first experiment (Fig. 1), we were unable to detect PGI<sub>2</sub> production by indomethacin-treated aortas incubated in PRP or PPP. Obviously, the incubation conditions in our last experiment (temperature 37°C instead of room temperature; time 10 instead of 3 min; plasma mixing by stirring instead of shaking) enhanced the removal of indomethacin from vessel wall cyclo-oxygenase.

Later experiments, to be presented elsewhere, showed that reactivation of indomethacin-blocked vascular cyclo-oxygenase needs about 6 min of incubation in plasma at 37°C, stirred at 1,000 r.p.m. The presence of platelets during this incubation had no significant effect.

Thus, we have shown that in a variety of conditions blood platelets do not enhance vascular prostacyclin production by supplying cyclic endoperoxides. Experiments suggestive of the existence of such a pathway did not take into account that the binding of indomethacin to cyclo-oxygenase, and consequently, the cyclo-oxygenase inactivation is reversible and can easily be overcome by incubation of indomethacin-treated tissue in an indomethacin-free medium.

*Note added in proof:* Recently, Needleman *et al.*<sup>12</sup> reached a similar conclusion, namely that in normal conditions blood vessels do not use platelet cyclic endoperoxides for prostacyclin synthesis.

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## Is alanine the active component in anterior pituitary extracts proposed to contain a thymotropic factor?

SAXENA AND TALWAR have reported the occurrence of a factor in extracts of the anterior pituitary which stimulates thymidine incorporation into thymocytes *in vitro*<sup>1</sup>. The possible existence of a previously unknown thymotropic pituitary factor was discussed. The test system used by Saxena and Talwar consisted of isolated rat thymocytes cultured in a defined glucose salt medium without serum or other additives. Anterior pituitary extract (APE) was added and the uptake of <sup>3</sup>H-thymidine into acid-insoluble material during short-term incubation was greatly stimulated. It was briefly mentioned that stimulation was also obtained when thymocytes were cultured in RPMI or Parker 199 medium. No effect was observed on tissue slices from liver, heart, kidney and diaphragm. The factor was said to have a molecular weight of 500 and an isoelectric point of 7.9, but the methods of determining these properties were not described. We report here on the isolation of an active component in the APE and its identification as the amino acid alanine. APE was tested for the ability to stimulate thymidine incorporation and mitotic activity in guinea pig thymocytes in our *in vitro* systems (test systems are described in the legends to Table 1 and Fig. 1). The incorporation of thymidine and the mitotic activity were both stimulated in our experiments (Fig. 1a). The stimulatory effect on thymidine incorporation was dose dependent and varied between 129 and 156% in different experiments.

APE was purified using preparative thin layer chromatography (TLC). In the first system (cellulose; ethanol 95%: ammonium acetate 1 M, pH 5.0; 7:3), at least 10 separate ninhydrin-positive bands were obtained. Fractions were eluted according to the ninhydrin stain and tested for the ability to stimulate thymidine incorporation. Active material was rechromatographed in other solvent systems, fractionated and again tested for activity. A fraction which was considered homogeneous was on analytical TLC found to correspond to the amino acid alanine. When alanine was used as a reference in the preparative runs, all the activity was found in the region of this amino acid. (Amino acids mentioned are in the L form unless indicated otherwise.)

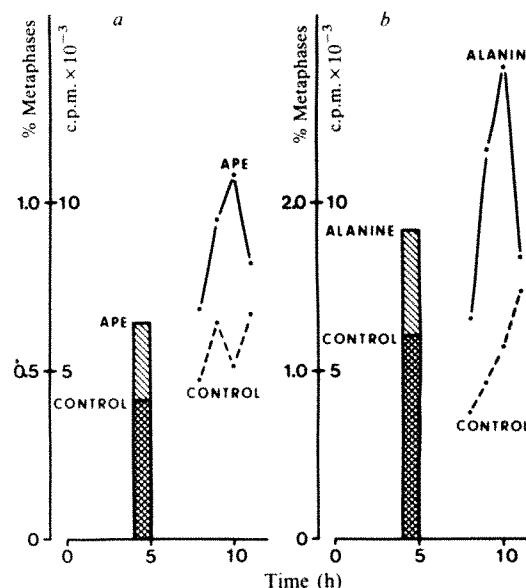
The RPMI 1640 medium, used by us and many others for lymphoid cell cultures, contains all common amino acids except alanine and cysteine<sup>2</sup>. It has recently been discovered that alanine, when added to RPMI 1640 medium, causes a quantitatively important increase in the thymidine incorporation into thymocytes and lymphocytes of different organs and species<sup>3</sup>. Isolated rat liver cells are not stimulated by alanine (O.S., unpublished results). The addition of alanine also increases the frequency of mitoses in cultures of guinea pig thymocytes (Fig. 1b and ref. 4). The stimulatory effect of alanine in RPMI 1640 medium is detected at a concentration of 10  $\mu$ M and is maximal at 100  $\mu$ M. Concentrations up to 2 mM cause the same maximal stimulation. D-Alanine or  $\beta$ -alanine do not stimulate thymidine incorporation. Alanine is one of the most common amino acids in serum<sup>5</sup> and the alanine requirement would probably be provided for by the addition of serum to the culture medium. However, uncontrolled factors such as general enzyme inhibitors and uncharacterised growth factors present in serum render its use unsuitable in studies of lymphoid growth factors.

Lymphoid cells from different species seem to vary in sensitivity to alanine stimulation. In guinea pigs, for example, thymocytes are stimulated by less than 200%, whereas human thymocytes could be stimulated by more than 350% in RPMI 1640 medium (O.S., unpublished results).

Saxena and Talwar<sup>1</sup> used a defined glucose salt medium for their thymocyte cultures. We studied the effect of supplementing such a medium with nutrients, such as amino acids and vitamins. Table 1 compares the radioactivity incorporated into

thymocytes cultured in RPMI 1640 medium, in RPMI 1640 supplemented with alanine, and in Hank's-Dulbecco (H-D) medium (a mixture of equal parts of Hank's balanced salt solution and Dulbecco's phosphate-buffered saline, the compositions of which are described in ref. 2). The highest incorporation is found in the alanine-supplemented RPMI 1640 medium and the lowest in the H-D medium. Table 1 also shows the effect of addition of APE or alanine to the different media. When APE was tested for activity in alanine-supplemented RPMI 1640 medium, no stimulation of the thymidine incorporation was obtained. Thus, the stimulatory effect of APE in the RPMI 1640 medium depends on its alanine content. Material in extracts from bovine liver, spleen and thymus, which stimulated the thymidine incorporation into lymphocytes from various sources, has earlier been identified as alanine<sup>3</sup>. As shown in Table 1, addition of alanine is without stimulatory effect on thymidine incorporation in the H-D medium, indicating that the stimulatory effect of APE in this medium depends on alanine in combination with other amino acids, probably present in the extract. (Crude low molecular weight material (<10,000 daltons) from bovine thymus stimulates thymidine incorporation into guinea pig thymocytes cultured in H-D medium (O.S., unpublished data). Even highly purified low molecular weight material from tissue extracts (thymus, spleen and liver) contains, in addition to alanine, various amounts of other amino acids (K. Nordlind, personal communication).

We conclude that the stimulatory effect of APE on thymidine incorporation and the frequency of mitoses in RPMI 1640



**Fig. 1** Effects of APE (a) and alanine (b) on incorporation of <sup>3</sup>H-thymidine and frequency of colcemid-arrested metaphases in cultured thymus cells. The method for determination of <sup>3</sup>H-thymidine incorporation is described in the legend to Table 1. For quantification of mitotic activity, 1-ml cultures with  $5 \times 10^6$  cells were incubated in RPMI 1640 medium at 37°C. After 30 min, APE (1:2; see Table 1) or alanine (to make 0.65 mM) was added (time 0) in a volume of 0.2 ml. The procedure for preparation of the cells for mitotic counts is described elsewhere<sup>4</sup>. Briefly, cells were swelled on glass slides by hypotonic treatment, fixed by addition of methanol:acetic acid (3:1) and stained; 5,000 cells from each group were examined at  $\times 1,000$  magnification. Incorporation of <sup>3</sup>H-thymidine (c.p.m.) in 1 h is shown by vertical bars, and mitotic activity (% metaphases) by continuous (APE or alanine) and dashed lines (controls). Each point on the curves represents the frequency of cells in metaphase after a 2-h pulse with colcemid (100  $\mu$ l added to make 0.08  $\mu$ g ml<sup>-1</sup>). The values for <sup>3</sup>H-thymidine incorporation and mitotic activity after addition of APE (but not alanine) were measured at the same time using cells from the same suspension in both cases.

**Table 1** Thymidine incorporation into guinea pig thymocytes cultured in different media, and the effect of addition of APE or alanine

Addition	Incorporated radioactivity (c.p.m., mean of duplicate cultures)		
	RPMI 1640	RPMI 1640 + alanine (0.5 mM)	Hanks-Dulbecco
Control (sterile water)	7,602	12,446	4,033
Alanine (50 nmol per culture)	11,712 (154)	12,538 (101)	3,206 (79)
APE 1:2	10,381 (137)	10,274 (83)	5,530 (137)
1:4	9,905 (131)	11,114 (89)	5,294 (131)
1:6	8,505 (112)	10,861 (87)	4,771 (118)

$5 \times 10^5$  guinea pig thymocytes were incubated in 0.1-ml samples of the media indicated above. Incubations were carried out in a Linbro micro-titration plate in  $37^\circ\text{C}$  in an atmosphere of 10%  $\text{CO}_2$  in air. Penicillin ( $100 \text{ IU ml}^{-1}$ ) and streptomycin ( $100 \mu\text{g ml}^{-1}$ ) were added to all media and glutamine (2 mM) was added to the RPMI media before the onset of cultures. One ampulla of lyophilised APE was dissolved in 5 ml sterile water (according to instructions from R. K. Saxena) and diluted as indicated above; 10  $\mu\text{l}$  of APE or alanine was added to duplicate cultures from the start. Control cultures received 10  $\mu\text{l}$  of sterile water. Tritiated thymidine (0.5  $\mu\text{Ci}$ , 1  $\mu\text{M}$ ) in 10  $\mu\text{l}$  physiological saline was added after 4 h. One hour later the cultures were collected on a Skatron multiple cell collector using glass fibre filters. Incorporated radioactivity was measured in a Packard liquid scintillation spectrometer. Values in parentheses show the percentage of incorporation in the different media after the addition of alanine or APE compared with the control cultures.

medium are due to the presence of alanine in the APE. The effect of APE in glucose salt medium, shown by Saxena and Talwar, is probably caused by the presence of several essential nutrients in the APE, for example, a mixture of amino acids. As shown here, the growth of thymic cells (and probably other cells also) in short-term cultures is very dependent on the presence of essential nutrients. When culturing lymphoid cells, alanine should be included in the culture medium.

Due to the facts presented above, we feel that the existence of a new thymotrophic pituitary factor is not yet fully confirmed.

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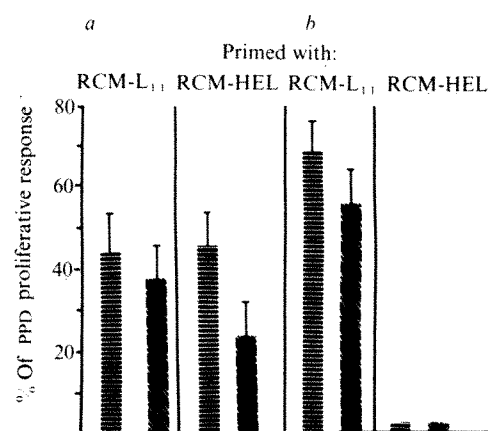
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## Amputation of a suppressor determinant on lysozyme reveals underlying T-cell reactivity to other determinants

THE response to several thymus-dependent antigens is influenced by immune response (*Ir*) genes, usually those of the major histocompatibility complex. However, the relevant gene products and their cellular sites of action have not been fully characterised. It is evident that in certain cases, the *Ir* genes can

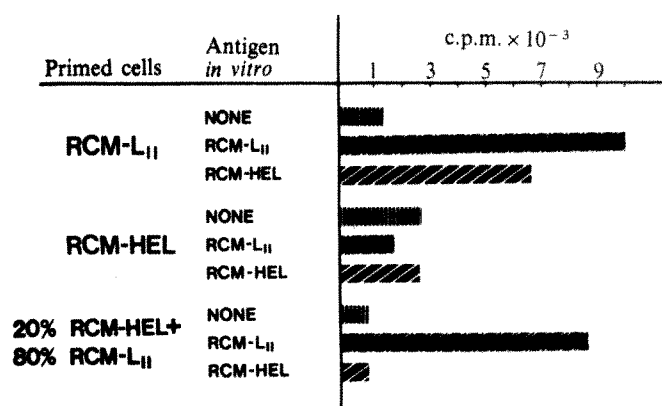
function to select those determinants which will be presented to activate T cells. This control operates, at least in part, at the level of macrophage-T-cell interactions. We have previously proposed<sup>1</sup> that the failure of a T-cell response in H-2<sup>b</sup> mice following immunisation with chicken lysozyme (HEL) could be due to preferential stimulation of suppressor T cells. A suppressor mechanism which nullifies the activity of lysozyme-specific T-helper cells has been reported from our laboratory<sup>2</sup>. Comparison of the amino acid sequence of various closely related lysozymes reveals that molecules which have tyrosine at position 3 are immunogenic in H-2<sup>b</sup> mice whereas lysozymes with phenylalanine at position 3 are non-immunogenic in such strains<sup>3</sup>. We have considered the possibility that the latter lysozymes possess a determinant that preferentially stimulates suppressor cells in H-2<sup>b</sup> mice, whereas the former lack this 'suppressor determinant'. Other studies<sup>4-6</sup>, including those by Schwartz *et al.*<sup>7</sup> and Turkin and E.E.S.<sup>8</sup>, support a phenomenon of generalised suppression caused by restricted determinants. Here, we demonstrate that a strain which is unresponsive to HEL and whose T-cell proliferative activity is absent following immunisation with the entire molecule, possesses the potential to generate a vigorous T-cell response directed against a large fragment of the antigen. Suppressor cells directed against a restricted determinant on the molecule obscure this competence to respond to the majority of the molecule.

As a first test of the proposition that HEL carries a suppressor determinant, a major peptide of HEL lacking the putative



**Fig. 1** Groups of 10 C57BL/10 (a) and 10 C57BL/10.A (b) mice were injected with either 15  $\mu\text{g}$  of RCM-L<sub>11</sub> or 22  $\mu\text{g}$  RCM-HEL, emulsified in complete Freund's adjuvant (containing *Mycobacterium tuberculosis* strain H37Ra), divided in the hind footpads. Four weeks later, peritoneal exudate T-enriched lymphocytes (PETLES) were prepared as described by Schwartz *et al.*<sup>10</sup>. Five days after injection of 1 ml of 10% thioglycollate, the peritoneal cells were recovered, washed and passed over nylon wool to enrich for T cells<sup>11</sup>.  $2 \times 10^5$  PETLES per well were stimulated in round-bottomed microtitre plates (Microbiological Associates) with medium alone (modified Eagle-Hanks' medium containing fresh L-glutamine, 2-mercaptoethanol, antibiotics and 10% heat-inactivated fetal bovine serum), or supplemented with 50  $\mu\text{g ml}^{-1}$  purified protein derivative (PPD), or antigens (4-200  $\mu\text{g ml}^{-1}$ ). Cultures were incubated at  $37^\circ\text{C}$  in 2%  $\text{CO}_2$ , 98% air for 5 d. Four days after initiation, cultures were pulsed with 1  $\mu\text{Ci}$  of [Methyl-<sup>3</sup>H]thymidine (specific activity 6.7 Ci mmol<sup>-1</sup>, NEN) in 10  $\mu\text{l}$  phosphate-buffered saline. The amount of antigen-stimulated incorporation of label into cellular DNA was determined by counting the cells collected 16 h later on filter disks in a liquid scintillation counter. The maximum stimulation observed in three separate experiments has been plotted as a relative per cent of the response stimulated by PPD (purified protein derivative of the same strain of *M. tuberculosis*). PPD stimulation ranged from 25-50,000 c.p.m. in different experiments; the background (medium alone) was 500-2,000 c.p.m. The bars represent mean incorporation  $\pm$ s.e.m. The antigen used in culture was either RCM-L<sub>11</sub> (▨) or RCM-HEL (■).





**Fig. 2** Using an identical protocol to that described in the legend to Fig. 1, groups of B10 mice were primed with 15  $\mu$ g RCM-L<sub>II</sub> or 22  $\mu$ g RCM-HEL, and the PETLES were isolated 4 weeks later. The individual or mixed PETLES populations were stimulated as shown, with 50  $\mu$ g ml<sup>-1</sup> RCM-L<sub>II</sub> or 75  $\mu$ g ml<sup>-1</sup> RCM-HEL. Mixtures of 2%, 20% or 50% RCM-HEL-primed PETLES with RCM-L<sub>II</sub>-primed PETLES were used, resulting in 22, 10 and 25% of the RCM-L<sub>II</sub>-induced level of incorporation, respectively. A representative experiment is shown, in which only the 20% mixture results are plotted.

suppressor determinant was used to prime B10 and B10.A congenic mice, and the *in vitro* proliferative potential of these peptide-primed T cells was determined. The peptide used was the reduced and carboxymethylated, cyanogen-bromide peptide RCM-L<sub>II</sub> (amino acid residues 13–105). This is the largest peptide derivable from HEL which lacks the presumptively suppressive region around the N-terminus. As this peptide was obtained from RCM-HEL, one underlying assumption that had to be tested was that the T-cell cross-reactivity observed between HEL and RCM-HEL<sup>9</sup> extended to the suppressor cell level. Therefore, groups of B10 and B10.A mice were immunised with intact RCM-HEL and the proliferative responses of T cells from these mice were determined in the peritoneal exudate T-enriched lymphocyte (PETLES) system first developed by Schwartz *et al.*<sup>10</sup>. Whereas RCM-HEL induced a vigorous PETLES proliferative activity in the B10.A strain, there was no response to RCM-HEL by B10 PETLES. We then tested the prediction that B10 mice should be capable of responding to RCM-HEL following 'amputation' of the suppressor-inducing determinant. B10 and B10.A mice were immunised with 15  $\mu$ g of RCM-L<sub>II</sub> (a molar equivalent of the standard dose of intact RCM-HEL) in complete Freund's adjuvant. Three to six weeks later, PETLES were prepared and the proliferative response of these cells was determined. As shown in Fig. 1, both B10 and B10.A PETLES from mice primed with RCM-L<sub>II</sub> demonstrate active proliferative responses to this large peptide. Furthermore, the stimulatory determinants recognised on RCM-L<sub>II</sub> are also available on intact RCM-HEL, as shown by the high degree of cross-stimulation of RCM-L<sub>II</sub>-primed PETLES in the B10.A case. In contrast, the distinct preference for RCM-L<sub>II</sub> by B10 PETLES may reflect the effect of primary suppressor T-cell recognition of the suppressor determinant on intact RCM-HEL.

The functional inability of RCM-HEL-primed B10 PETLES to proliferate in the presence of strong responsiveness to determinants on RCM-L<sub>II</sub> can be attributed to a suppressor-inducing determinant present on RCM-HEL, but absent from RCM-L<sub>II</sub>. Taken together, the regions absent from RCM-L<sub>II</sub> (residues 1–12 and 106–120) represent a determinant region very similar to the N–C disulphide peptide. This peptide has previously been shown to induce HEL-specific suppressor cells in B10 mice<sup>1</sup>. The combined evidence supports the concept that there is a population of precursor T cells available in B10 mice which can be effectively stimulated by determinants on RCM-L<sub>II</sub>.

The implication that RCM-HEL is able to raise suppressor T cells which can affect the proliferative response to RCM-L<sub>II</sub> was then tested directly in the experiment shown in Fig. 2. RCM-L<sub>II</sub>-primed PETLES, when stimulated by homologous peptide in culture, gave a proliferative response that was 55% of the value induced by purified protein derivative (PPD). RCM-HEL-primed PETLES again showed no proliferation *in vitro*, when stimulated by either RCM-HEL or RCM-L<sub>II</sub>, suggesting that no expansion of RCM-L<sub>II</sub>-specific T cells had occurred. These putatively suppressive cells were then titrated into the responsive, RCM-L<sub>II</sub>-primed population. As shown in Fig. 2, 20% of the RCM-HEL-primed PETLES completely obliterate the specific response; actually, this was true even when the proportion of suppressive cells was only 2%. It is particularly interesting that the suppression only occurred over an antigenic bridge which linked the suppressor determinant with the proliferation-inducing determinant. Thus, when suppressor cells were present and potentially fully active, RCM-L<sub>II</sub> added in culture stimulated a response similar to that seen in the absence of suppressor T cells. However, with RCM-HEL as antigen, suppression was easily demonstrable. This dominant suppressive effect obviated the possibility that for some reason, RCM-HEL was catabolised too quickly in B10 mice to make an impression on the immune machinery.

This reasonably active suppression on a primed T-cell proliferative response has previously been difficult to observe. It suggests that the most suitable system in which to detect suppression may be one, as above, in which the antigen (fragment) stimulating the target response, here RCM-L<sub>II</sub>, does not itself act on suppressor cells.

We conclude that many situations arise in systems under obvious genetic control<sup>2,7</sup>, but also with more complex antigens, where a particular haplotype will regard a determinant region as suppressive. The existence of this recognition will then obscure an underlying, vigorous responsiveness directed against other parts of the molecule.

One, if not the major role, of *Ir* genes may be performed by antigen-presenting cells (macrophages) in selecting the appropriate determinant to be offered to different subpopulations of T cells<sup>12,13</sup>. Although this has widely been considered as a problem relating to the activation of helper cells, our results indicate that suppressor cell triggering is the critical factor for the outcome of the total response. Whether antigen presentation to suppressor cell precursors is a macrophage function remains to be established. Our work suggests that what occurs is the presentation of different determinants to the competing subpopulations of regulatory T cells.

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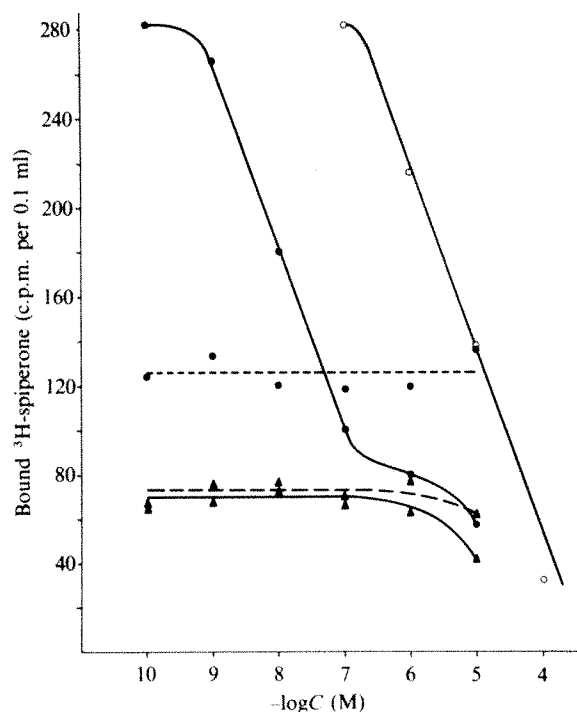
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## Solubilisation of high-affinity dopamine receptors

NEUROLEPTIC drugs are thought to act by blocking brain dopamine receptors<sup>1</sup>. *In vitro* binding assays using either <sup>3</sup>H-haloperidol<sup>2,3</sup> or <sup>3</sup>H-spiperone<sup>4,5</sup> have provided more direct evidence of this interaction. Although spiperone has been reported to label serotonergic receptors in the frontal cortex<sup>6</sup>, in the rat striatum, in both *in vitro*<sup>4-9</sup> and *in vivo* conditions<sup>5,10,11</sup>, the binding characteristics of this drug are dopaminergic. Initial attempts to solubilise the neuroleptic receptor were recently undertaken in our laboratory, but the <sup>3</sup>H-spiperone macromolecular complex obtained from rat striatal membranes after digitonin treatment<sup>12</sup> did not show the original high-affinity binding characteristics for dopamine agonists and antagonists except for spiperone itself. More recently, solubilised neuroleptic sites were found in digitonin extracts from calf caudate but they were still not characterised<sup>13</sup>. We now report the solubilisation of binding sites from dog striatum which retain the characteristics of membrane-bound dopamine receptors.

Mongrel dogs were anaesthetised with pentobarbital and Wistar rats were decapitated; their brains were removed and the striatum, cerebellum and frontal cortex were dissected out and homogenised in 0.25 M sucrose. A microsomal (P) fraction was prepared as previously described<sup>4</sup>. This membrane preparation was treated at 0 °C for 15 min with 1% digitonin suspended in 2 or 4 volumes of 0.25 M sucrose containing 10 mM sodium phosphate buffer (pH 7.2) and 0.01% NaN<sub>3</sub>. After centrifugation at 120,000 g (*r*<sub>av</sub>) for 60 min in a SW 65 Ti Spinco rotor, the supernatant, which was considered as the soluble preparation (~2 mg protein ml<sup>-1</sup>), was removed very carefully without disturbing the pellet. An aliquot of this solubilised preparation was incubated at 0 °C for 16 h in the presence of  $2 \times 10^{-9}$  M <sup>3</sup>H-spiperone (specific activity 23.6 Ci mmol<sup>-1</sup>, NEN) and various concentrations of unlabelled drugs. Aliquots of 0.1 ml of the incubation mixture were layered on the top of a Sephadex G-50 Medium column (13 × 0.5 cm). Elution was carried out at 2 °C with 10 mM sodium phosphate (pH 7.2) containing 0.01% NaN<sub>3</sub>. Four-drop fractions were collected in scintillation



**Fig. 1** Inhibitory effect of (+)butaclamol (●—●) and (–)butaclamol (○—○) on the binding of <sup>3</sup>H-spiperone to solubilised material from dog striatum. Control experiments with (+)butaclamol used cerebellum extract (▲—▲) and thermally inactivated (56 °C for 10 min) preparations from dog striatum (○—○) and cerebellum (▲—▲). The values given are from one typical experiment.

vials and then counted for radioactivity. The gel-filtration procedure enabled the bound <sup>3</sup>H-spiperone macromolecular complex to be separated from the free drug, as described in more detail previously<sup>12,14</sup>.

Figure 1 shows that the <sup>3</sup>H-spiperone binding in the solubilised preparation from dog striatum revealed a very pronounced stereospecific effect; (+)butaclamol competed with the binding at a concentration 300 times lower than (–)butaclamol, the inactive enantiomer. The inhibitory effect of

**Table 1** Inhibition of <sup>3</sup>H-spiperone binding in solubilised and membrane preparations from dog and rat striatum

	Dog striatum			Rat striatum		
	Soluble* A	Membrane B	A/B	Soluble* C	Membrane† D	C/D
Spiperone	$5.1 \times 10^{-9}$	$1.8 \times 10^{-9}$	2.8	$5.0 \times 10^{-9}$	$1.3 \times 10^{-9}$	3.8
Benperidol	$1.2 \times 10^{-8}$	$5.1 \times 10^{-9}$	2.4	$1.7 \times 10^{-5}$	$4.5 \times 10^{-9}$	3,778
Lysuride	$1.3 \times 10^{-8}$	$6.0 \times 10^{-9}$	2.2		$5.0 \times 10^{-9}$	
(+)Butaclamol	$2.0 \times 10^{-8}$	$1.5 \times 10^{-8}$	1.3	$6.2 \times 10^{-6}$	$2.0 \times 10^{-8}$	310
Haloperidol	$3.7 \times 10^{-8}$	$1.6 \times 10^{-8}$	2.3	$8.5 \times 10^{-6}$	$2.0 \times 10^{-8}$	425
Fluspirilene	$4.0 \times 10^{-8}$	$4.0 \times 10^{-8}$	1.0	$7.1 \times 10^{-7}$	$2.3 \times 10^{-8}$	31
Flupenthixol	$1.3 \times 10^{-7}$	$4.0 \times 10^{-8}$	3.3	$2.0 \times 10^{-5}$	$5.9 \times 10^{-8}$	339
(±)2-(N,N-Dipropyl) amino-5,6-dihydroxytetralin	$4.8 \times 10^{-7}$	$1.1 \times 10^{-7}$	4.4		$1.3 \times 10^{-7}$	
Chlorpromazine	$5.4 \times 10^{-7}$	$4.2 \times 10^{-8}$	13		$4.0 \times 10^{-8}$	
Fentanyl	$1.4 \times 10^{-6}$	$3.2 \times 10^{-5}$	0.04	$1.4 \times 10^{-4}$	$1.0 \times 10^{-4}$	1.4
Sulpiride	$1.6 \times 10^{-6}$	$5.6 \times 10^{-7}$	2.9		$8.7 \times 10^{-7}$	
Pipamperone	$2.9 \times 10^{-6}$	$1.3 \times 10^{-6}$	2.2		$1.3 \times 10^{-5}$	
Dextetimide	$5.0 \times 10^{-6}$	$2.6 \times 10^{-5}$	0.19	$1.7 \times 10^{-4}$	$3.2 \times 10^{-5}$	5.3
Mianserin	$5.3 \times 10^{-6}$	$4.2 \times 10^{-5}$	0.13		$2.0 \times 10^{-6}$	
(–)Butaclamol	$5.5 \times 10^{-6}$	$1.3 \times 10^{-5}$	0.42	$3.4 \times 10^{-5}$	$2.1 \times 10^{-6}$	16
R 5260	$4.5 \times 10^{-5}$	$\geq 10^{-4}$		$8.9 \times 10^{-7}$	$2.0 \times 10^{-5}$	0.04
Propranolol	$7.6 \times 10^{-5}$	$7.1 \times 10^{-5}$	1.1	$1.3 \times 10^{-4}$	$>10^{-4}$	
Atropine	$2.3 \times 10^{-4}$	$>10^{-4}$			$>10^{-4}$	
Diazepam	$2.5 \times 10^{-4}$	$1.3 \times 10^{-4}$	1.9	$>10^{-4}$	$>10^{-4}$	

Inhibition was measured at five different concentrations and expressed as the IC<sub>50</sub> value (the drug concentration causing 50% inhibition of the stereospecific <sup>3</sup>H-spiperone binding). The blank values were obtained using  $2 \times 10^{-6}$  M (+)butaclamol<sup>4</sup> except for the soluble preparation of rat striatum where the heat-inactivated preparation was used as blank<sup>12</sup>.

\*For spiperone, (+)butaclamol, haloperidol, flupenthixol, fentanyl and mianserin, the IC<sub>50</sub> values in solubilised dog striatum preparations are the mean values to two to five different experiments. This was also the case for the spiperone, benperidol, (+)butaclamol and (–)butaclamol IC<sub>50</sub> values in solubilised preparations from rats.

†Most of the values were taken from refs 4 and 6.

(+)-butaclamol was not observed when a cerebellum extract or a thermally inactivated preparation from striatum and cerebellum was used. The stereospecificity of the solubilised binding sites is quite comparable to that found in the membrane preparations from rat<sup>4,7-9</sup> and dog striatum (Table 1). When compared with membrane fractions, the solubilised preparation from dog striatum was more rapidly inactivated by heat<sup>15</sup>. Table 1 shows that various dopamine antagonists belonging to different chemical classes (butyrophenone, phenothiazine, diphenylbutylamine, thioxanthene, procainamide and benzoquinolizine) and dopamine agonists (tetraline derivative<sup>6</sup> and lysuride<sup>16</sup>) were nearly all of similar activity in soluble and membrane preparations from dog striatum (correlation coefficient  $r = 0.94$ ), whereas anticholinergic, analgesic, a  $\beta$ -blocking agent, a minor tranquilliser and serotonin antagonists were practically inactive. Of the neuroleptic drugs, only chlorpromazine was relatively less active in the solubilised preparation, presumably because of its high affinity for nonspecific binding sites<sup>17-19</sup>.

In contrast to this, the  $IC_{50}$  values obtained using a solubilised preparation from rat striatum differed markedly from that obtained in membrane preparations. For example, benperidol displayed a 3,800-fold difference between the  $IC_{50}$  values of soluble and membrane preparations in rat striatum and only a twofold difference in the dog. Note that the affinities of dopamine antagonists and agonists are quite similar in membrane preparations of dog and rat. Interestingly, R 5260 (compound 25 of ref. 20), which is an analgesic ( $IC_{50}$  of fentanyl binding  $1.0 \times 10^{-9}$  M), was more active in inhibiting  $^3H$ -spiperone binding in the rat striatum soluble preparation than potent neuroleptic drugs like haloperidol and benperidol. Therefore, the binding sites solubilised from rat striatum displayed only a high affinity for spiperone or derivatives related to the spiro moiety (R 5260 and also compounds 2, 3, 4 of ref. 20). We assume that such binding sites correspond to the non-stereospecific displaceable sites detected in rat striatum using  $^3H$ -spiperone as ligand<sup>21</sup>.

As shown in Fig. 2, a good correlation between the spiperone binding in solubilised preparations and the antagonism of apomorphine-induced emesis was found for the dog striatum ( $r = 0.89$ ) but not for the rat striatum. Spiperone was the only drug equiactive in solubilised preparations from both animals. Previous investigations clearly demonstrated that the haloperidol and spiperone binding of neuroleptic drugs correlated well with their pharmacological activities in the apomorphine test in rat<sup>6</sup> and dog<sup>20,22,23</sup> and their clinical potency<sup>17</sup>.

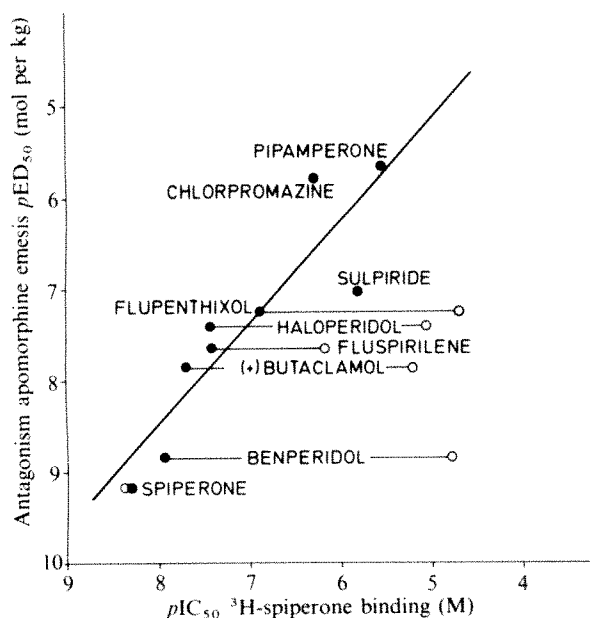


Fig. 2 Correlation between the  $IC_{50}$  values of  $^3H$ -spiperone binding in solubilised preparations from dog (●) and rat (○) and the antagonism of apomorphine-induced emesis in dog (see refs 22, 23).

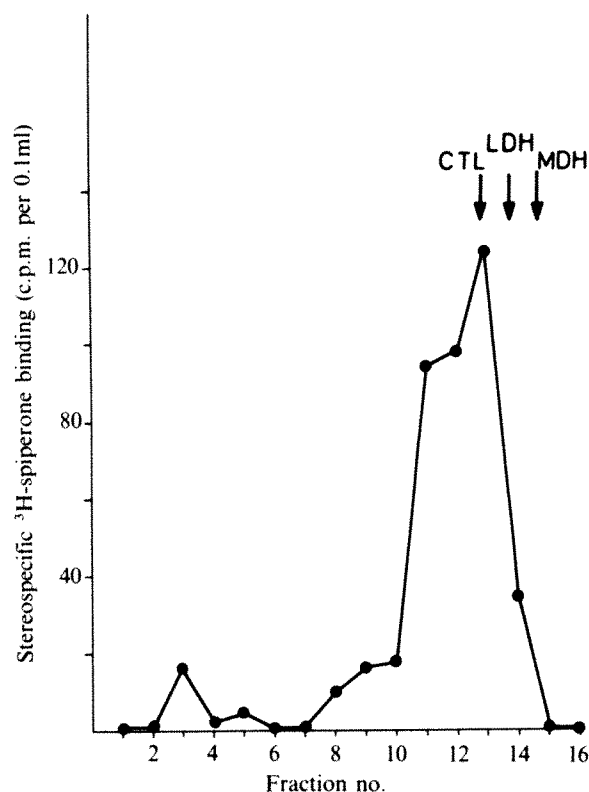


Fig. 3 Distribution of stereospecific  $^3H$ -spiperone binding after centrifugation of a digitonin extract from dog striatum. 1 ml of soluble extract was layered on 12 ml sucrose gradient (15–30%) buffered with 10 mM sodium phosphate (pH 7.2) containing 0.03% digitonin and 0.01%  $Na_3$ . Centrifugation was run at 2–3 °C in SW 40 Ti rotor (Spinco) at 30,000 r.p.m. (111,700g,  $r_{av}$ ) for 16 h. Marker enzymes: catalase (CTL), lactate dehydrogenase (LDH) and malate dehydrogenase (MDH).

Stereospecific  $^3H$ -spiperone binding in soluble dog striatum preparations was found to be saturable ( $K_{D(app)}$  at 0 °C = 4.8 nM) and reversible ( $t_{1/2}$  dissociation at 5 °C = 49 min). A more detailed kinetic analysis will be presented elsewhere<sup>15</sup>. To assess the macromolecular nature of these binding sites, the soluble extract from dog striatum was submitted to sedimentation centrifugation through a sucrose gradient and then compared with the sedimentation of marker enzymes. The peak of binding sites seemed to migrate more rapidly than malate dehydrogenase (4.3S) and lactate dehydrogenase (7.0S), but more closely resembled that of catalase (11.3S). When compared with the sedimentation properties of muscarinic receptor<sup>14,15</sup>, the spiperone binding sites seemed to be more heterogeneous and to have a higher molecular weight (> 200,000).

The present results provide evidence that a macromolecular complex, able to bind  $^3H$ -spiperone stereospecifically, was solubilised from dog striatum. Most of the solubilisation criteria were fulfilled: not sedimentable at 262,000g ( $r_{av}$ ) for 60 min, absence of lamellar membrane structure in electron microscopy<sup>24</sup>, and low Svedberg coefficient compared with that of membranes.

Four lines of evidence indicate that the spiperone binding solubilised by digitonin from dog striatum and characterised by gel-filtration or sedimentation gradient is of dopaminergic nature. First, it was localised in the striatum but not in the cerebellum, and preliminary results showed that the binding sites solubilised from frontal cortex displayed a higher affinity for serotonin antagonists and a lower affinity for dopamine antagonists (in preparation). Second, in contrast to findings in the rat striatum, various dopamine antagonists from different chemical classes, chosen to display a wide range of activity, were nearly equiactive in the soluble and membrane preparation from dog striatum. Serotonin antagonists like pipamperone and mianserin were very poor inhibitors of  $^3H$ -spiperone binding to



soluble sites from striatum, whereas both drugs were very active in the frontal cortex<sup>6</sup>. Third, a tetraline derivative, known to be the most specific dopamine agonist<sup>6,25</sup>, and thus being inactive in the spiperone binding assay in the frontal cortex, inhibited the spiperone binding of the solubilised preparation of dog striatum at very low concentrations. Finally, the good correlation between the inhibitory effects of drugs in the soluble preparation and their antagonism of apomorphine-induced emesis is only possible if the relative high affinity of the dopamine receptors is maintained after the solubilisation process.

Thus, the above results provide evidence that the spiperone binding sites solubilised by digitonin treatment from dog striatum retain the characteristics of dopamine receptors.

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## Melanotropin potentiating factor is the C-terminal tetrapeptide of human $\beta$ -lipotropin

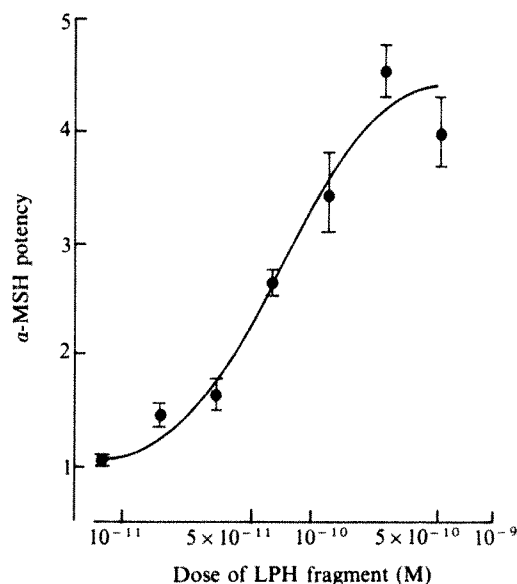
WE recently demonstrated that the molar pigmentary potency of human  $\beta$ -lipotropin ( $\beta$ -LPH) is greater than other melanocyte-stimulating hormones (MSHs) on *Anolis* skin; it is 2.6–4.0 times greater than  $\alpha$ -MSH<sup>1</sup> using the *Anolis* rate method of MSH bioassay<sup>2</sup>. We also found (unpublished) human  $\beta$ -LPH to be 2 to 4 times more potent than  $\alpha$ -MSH using the steady state (quantal<sup>3</sup>) method. This was due to a potentiation of the MSH sequence of  $\beta$ -LPH (LPH<sub>47–53</sub>) by a factor associated with  $\beta$ -endorphin (LPH<sub>61–91</sub>). We showed that the melanotropin potentiating factor (MPF) was not the opiate peptide, <sup>5</sup>Met-enkephalin (LPH<sub>61–65</sub>)<sup>4</sup>, and now report the identification of MPF as the sequence LPH<sub>88–91</sub>.

**Table 1** Intrinsic molar potencies of LPH peptide fragments calculated relative to  $\alpha$ -MSH

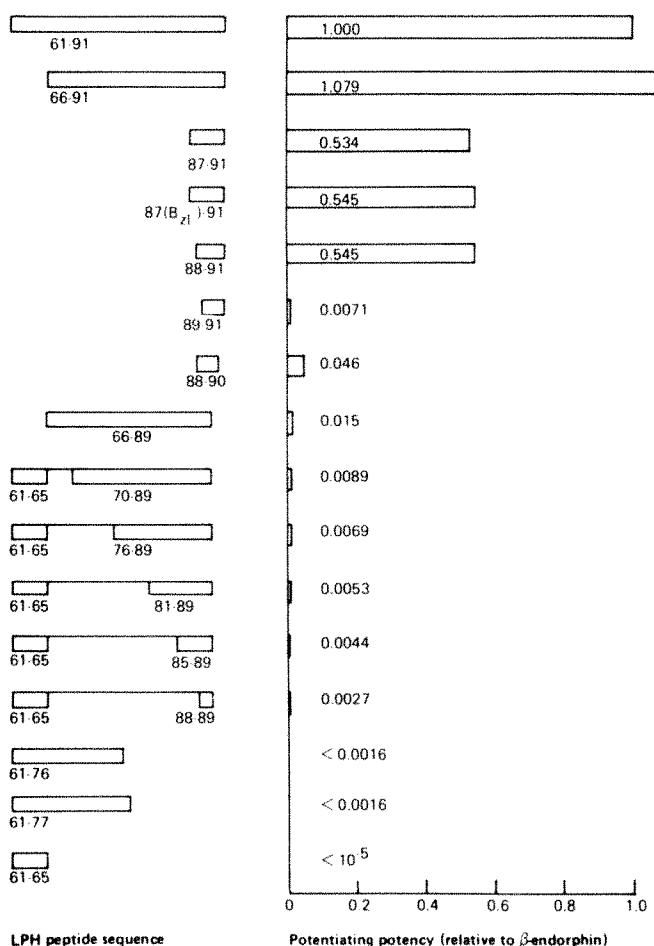
LPH Structure ( $\alpha$ -MSH)	Potency (1.000)
61–91	$5.036 \times 10^{-4}$ (4.813 and $5.270 \times 10^{-4}$ )
66–91	$3.590 \times 10^{-4}$ (3.406 and $3.785 \times 10^{-4}$ )
87–91	$1.409 \times 10^{-4}$ (1.377 and $1.442 \times 10^{-4}$ )
87(Bzl)–91	$1.667 \times 10^{-4}$ (1.574 and $1.765 \times 10^{-4}$ )
88–91	$3.245 \times 10^{-5}$ (3.067 and $3.434 \times 10^{-5}$ )
89–91	$4.427 \times 10^{-5}$ (4.101 and $4.780 \times 10^{-5}$ )
88–90	$4.266 \times 10^{-5}$ (4.027 and $4.520 \times 10^{-5}$ )
66–89	$2.967 \times 10^{-4}$ (2.797 and $3.148 \times 10^{-4}$ )
61–65, 70–89(D-Ala <sup>62</sup> , Leu <sup>65</sup> )	$2.548 \times 10^{-4}$ (2.432 and $2.670 \times 10^{-4}$ )
61–65, 76–89(D-Ala <sup>62</sup> , Leu <sup>65</sup> )	$2.105 \times 10^{-4}$ (1.992 and $2.225 \times 10^{-4}$ )
61–65, 81–89(D-Ala <sup>62</sup> , Leu <sup>65</sup> )	$8.588 \times 10^{-5}$ (8.269 and $8.920 \times 10^{-5}$ )
61–65, 85–89(D-Ala <sup>62</sup> , Leu <sup>65</sup> )	$7.586 \times 10^{-5}$ (7.205 and $8.199 \times 10^{-5}$ )
61–65, 88–89(D-Ala <sup>62</sup> , Leu <sup>65</sup> )	$5.237 \times 10^{-5}$ (4.921 and $5.573 \times 10^{-5}$ )
61–76	$2.245 \times 10^{-5}$ (2.095 and $2.406 \times 10^{-5}$ )
61–76 (D-Ala <sup>62</sup> , Leu <sup>65</sup> )	$8.878 \times 10^{-5}$ (8.375 and $9.412 \times 10^{-5}$ )
61–77	$2.245 \times 10^{-5}$ (2.095 and $2.406 \times 10^{-5}$ )
61–77 (D-Ala <sup>62</sup> , Leu <sup>65</sup> )	$6.140 \times 10^{-5}$ (5.884 and $6.408 \times 10^{-5}$ )
61–65	$6.890 \times 10^{-8}$ (6.764 and $7.017 \times 10^{-8}$ )

Dose-response curves were obtained to  $\alpha$ -MSH and the 18 peptide sequences, using the *Anolis* rate method of MSH bioassay<sup>2</sup>. Using analysis of variance<sup>8</sup>, the slope of the dose-response curves of the LPH peptide fragments did not differ significantly from that of  $\alpha$ -MSH ( $P > 0.05$ ). The 95% fiducial limits of the estimated potencies are given in parentheses.

The *Anolis* rate method of MSH bioassay<sup>2</sup> was used in these experiments. The peptides Tyr-Lys-Lys-Gly-Glu (human  $\beta$ -LPH<sub>87–91</sub>), its (tyrosyl) *O*-benzyl derivative [87(Bzl)-91], Lys-Lys-Gly-Glu (89–91), Lys-Gly-Glu (88–91), and Lys-Lys-Gly (88–90) were prepared by classical solution methods and characterised by TLC, paper electrophoresis and amino acid analysis of acid and/or enzymatic digests. Other peptides used in



**Fig. 1** The dose-related potentiation of  $\alpha$ -MSH potency by  $\beta$ -endorphin. Dose-response curves were obtained from eight two-fold dilution series of  $\alpha$ -MSH, seven of which had had added  $\beta$ -endorphin concentrations. The potencies of each  $\alpha$ -MSH dose-response curve in the presence of  $\beta$ -endorphin concentration was calculated relative to that in the absence of added  $\beta$ -endorphin. Thus each point on the graph represents the increase in  $\alpha$ -MSH potency with a  $\beta$ -endorphin concentration. The 95% fiducial limits of the estimated potencies are represented as vertical bars. There was a significant increase in  $\alpha$ -MSH potency with concentrations of  $16 \times 10^{-12}$  M  $\beta$ -endorphin or greater ( $P < 0.01$ ).



**Fig. 2** Potentiating activities of LPH fragments on  $\alpha$ -MSH potency. Bioassays of  $\alpha$ -MSH were performed with and without LPH peptide fragments in various concentrations. The dose-related potentiation of  $\alpha$ -MSH potency by each LPH fragment was measured as described in Fig. 1 and calculated relative to that of  $\beta$ -endorphin (LPH<sub>61-91</sub>) using a two-factorial assay system.

these experiments were prepared by N. N. Petter (ICI) by solid phase methods<sup>5</sup>.

Dose-response curves were obtained for  $\alpha$ -MSH and the synthetic LPH peptide fragments and their intrinsic molar potencies were calculated (Table 1). All showed negligible intrinsic MSH activity. Constant concentrations of the LPH peptide sequences were then incorporated into the twofold dilutions used to obtain the  $\alpha$ -MSH dose-response and their relative potencies were calculated.  $\beta$ -Endorphin potentiated  $\alpha$ -MSH potency with the dose-response curves remaining parallel. This potentiation was dose-related (Fig. 1) and the  $\alpha$ -MSH potency was increased to a maximum of 4.5-fold. To determine the sequence responsible for this potentiation, the potentiation activity of each peptide sequence was compared with that of  $\beta$ -endorphin (Fig. 2). Unequivocally, the results show that the sequence responsible for the potentiation is LPH<sub>88-91</sub>, Lys-Lys-Gly-Glu. Thus potentiating activity was abolished by the removal of the 88th or 91st amino acids from the tetrapeptide, while extension of the sequence to include tyrosine at position 87 did not increase potentiating activity further. The higher potentiating activity of LPH<sub>61-91</sub> ( $\beta$ -endorphin) and LPH<sub>66-91</sub> than LPH<sub>88-91</sub> was probably due to increased stability of the longer peptides as there was negligible activity in the sequences between LPH<sub>61</sub> and LPH<sub>89</sub>. These findings cannot be attributed to characteristics of the rate assay as we have obtained similar results (unpublished) with a steady state (quantal<sup>3</sup>) method. We therefore conclude that the melanotropic potentiating factor (MPF) is human LPH<sub>88-91</sub>, Lys-Lys-Gly-Glu. Whether MPF acts by stimulation of an independent receptor or by causing conformational changes is uncertain.

As a  $16 \times 10^{-12}$  M concentration of  $\beta$ -endorphin will potentiate MSH activity significantly (see legend to Fig. 1), the effect may be physiological. Although we have tested MPF activity on the *Anolis* skin, the unexpectedly high sebotropic<sup>6</sup> and neurotropic<sup>7</sup> potencies of  $\beta$ -LPH in the rat suggest that MPF may also modulate MSH peptide actions in the mammal and we are presently investigating this possibility.

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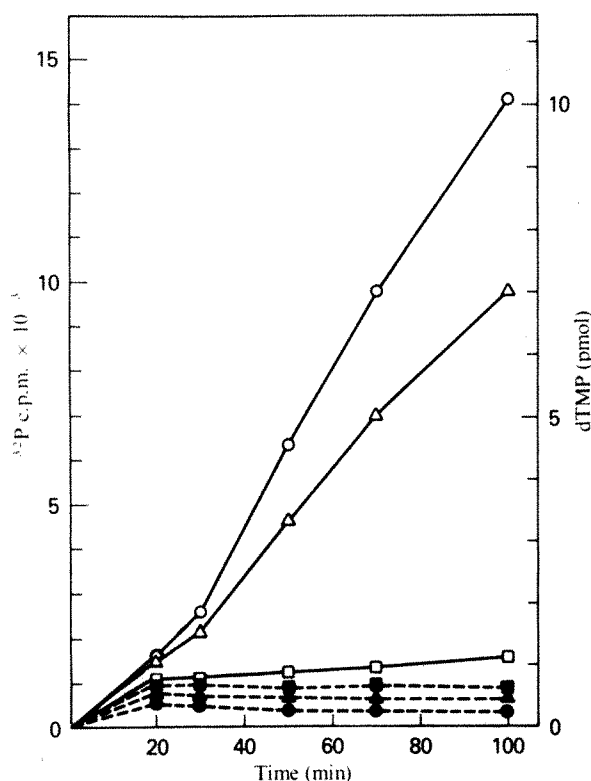
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## Inhibition of DNA synthesis *in vitro* by binding of benzo(a)pyrene metabolite diol-epoxide I to DNA

It has been shown that benzo(a)pyrene (BP) is a strong mutagen and carcinogen after metabolic activation by mixed function oxidases and epoxide hydratase. Evidence now indicates that ( $\pm$ )-7,8-dihydroxy-7,8,9,10-tetrahydrobenzo(a)pyrene (diol-epoxide I) is an ultimate carcinogenic and mutagenic form of BP<sup>1-4</sup>. This metabolic intermediate interacts covalently with nucleic acids<sup>5-8</sup>. Most binding to DNA, 80% of the total, occurs by coupling between the 2-amino group of guanine and the carbon 10 position of diol-epoxide I (ref. 5). Binding to adenine, less than 15% of the total, leads to partial denaturation of the DNA double helix<sup>6</sup>, and binding of diol-epoxide I to the phosphate groups of DNA results in DNA strand scission<sup>8</sup>. Intercalation of diol-epoxide I may cause conformational change of the DNA double helix<sup>6,7</sup>. Despite these extensive studies, the mechanism of alteration of the genetic function of DNA due to the binding of BP is not understood. We describe here a system which has enabled us to analyse the effect of BP binding to DNA on the replication of double-stranded circular DNA *in vitro*. pBR322 DNA (molecular weight,  $2.6 \times 10^6$ ) is an artificial plasmid DNA derived from ColE1 and pBR313 in *Escherichia coli*<sup>9</sup>. It carries the base sequence derived from ColE1 DNA at the site of origin of replication<sup>9</sup>. Like ColE1 DNA<sup>11-13</sup>, pBR322 DNA replicates semiconservatively and completely in a crude lysate of *E. coli*.

The binding of diol-epoxide I to pBR322 DNA was performed as described previously<sup>6</sup>. The number of diol-epoxide I molecules covalently bound to DNA, the molar ratio (MR), was determined using the radioactivity of <sup>14</sup>C-di-epoxide I (29.4 mCi mmol<sup>-1</sup>, NCI), UV absorption (254 nm), fluorescence and the molecular weight of pBR322 DNA. The covalent binding increased linearly as a function of increasing dose of diol-epoxide I concentration in the reaction mixture. High pressure liquid chromatography (Waters Bondapak C18



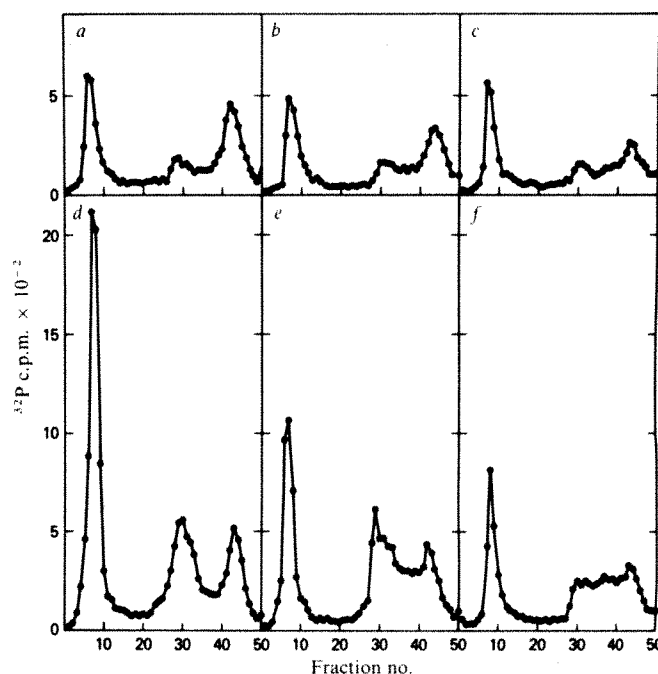
**Fig. 1** Assay of the incorporation of  $^{32}\text{P}$ -TMP into pBR322 DNA<sup>11,13</sup>. *E. coli* YS1 cells were grown in 600 ml OC medium enriched by 0.4% Bacto-Peptone (Difco) and 0.1% yeast extract (Difco). When the number of cells reached  $2 \times 10^8$  per ml, chloramphenicol was added ( $180 \mu\text{g ml}^{-1}$ ). The culture was then incubated for 2 h. The cells were washed with 50 mM potassium phosphate buffer (pH 7.4), suspended in 0.6 ml of 15% sucrose–75 mM potassium phosphate buffer (pH 7.4) and frozen in a dry ice–isopropanol bath. The crude cell extract was prepared by thawing cells in an ice–water bath, and the volume adjusted to 1.5 ml with 10% sucrose–50 mM phosphate buffer (pH 7.4). To the cell suspension 60  $\mu\text{l}$  2 M KCl, 150  $\mu\text{l}$  lysozyme ( $4 \text{ mg ml}^{-1}$ ) and 40  $\mu\text{l}$  5% Brij 58 were added at  $0^\circ\text{C}$  and mixed with a vortex mixer. After 30 min at  $0^\circ\text{C}$ , the mixture was centrifuged at 30,000 r.p.m. for 30 min in a Beckman SW 50.1 rotor. The standard assay mixtures (200  $\mu\text{l}$ ) were 25 mM potassium phosphate buffer, 67 mM KCl, 7.5 mM  $\text{MgCl}_2$ , 0.2 mM each of rNTPs, 25  $\mu\text{M}$  each of dNTPs, 2 mM spermidine, and contained 2  $\mu\text{g}$  pBR322 DNA,  $^{32}\text{P}$ -TTP (0.2  $\mu\text{Ci}$ ) and 40% cell extract. The assay was performed at  $30^\circ\text{C}$ . 30  $\mu\text{l}$  aliquots were taken at the times shown, precipitated with 10% trichloroacetic acid–0.1 M pyrophosphate, collected on filters (Whatman GF/C, 25 mm) and washed with 0.1 M HCl followed by ethanol. The open symbols indicate the standard assay mixture. Closed symbols indicate that rifampicin was added to the assay mixture. Control pBR322 DNA (MR = 0,  $\circ$ ,  $\bullet$ ); pBR322 DNA-bound diol-epoxide I (MR = 1.4,  $\triangle$ ,  $\blacktriangle$ ); pBR322 DNA-bound diol-epoxide I (MR = 5.8,  $\square$ ,  $\blacksquare$ ).

column,  $\text{H}_2\text{O}$  to 100% methanol gradient) of late elutant ( $\sim 570 \text{ ml}$ ) from Sephadex LH-20 column chromatography of enzymatically hydrolysed DNA supported our calculation of the MR (data not shown).

Incubation of unmodified pBR322 DNA (control) in an extract from *E. coli* cells resulted in the incorporation of  $^{32}\text{P}$ -TMP into the acid-precipitable fraction (Fig. 1). The incorporation increased linearly for at least 90 min. This incorporation was inhibited completely by the addition of rifampicin ( $25 \mu\text{g ml}^{-1}$ ) at zero time. Rifampicin inhibits RNA-primed initiation of DNA synthesis but does not affect the elongation of the deoxypolynucleotide chain<sup>11</sup>. When DNA covalently bound to diol-epoxide I (MR = 1.4) was used, the incorporation of  $^{32}\text{P}$ -TMP was reduced about 30% (Fig. 1). From MR = 0 to MR = 5.9 the rate of reduction was linear with the number of diol-epoxide I molecules bound to one pBR322 DNA molecule.

When MR = 5.9 the initial incorporation (up to 20 min) was normal, but the total  $^{32}\text{P}$ -TMP incorporation after 100 min was almost completely inhibited.

To study the mechanisms whereby DNA synthesis is inhibited by the binding of diol-epoxide I, we analysed the newly synthesised daughter strand DNA by sucrose density gradient centrifugation. The closed circular DNA synthesised *in vitro* appeared as a single peak with a skewed portion towards higher molecular weight in a neutral sucrose density gradient. Evidently the skewed portion represents closed circular DNA associated with newly synthesised DNA fragments. The amount of the closed circular DNA synthesised from DNA bound to diol-epoxide I (MR = 1.4) was about 30% less than that of the control (data not shown).



**Fig. 2** Analysis of newly synthesised DNA products in alkaline sucrose density gradient. Assays were performed as described in the legend to Fig. 1. Samples were taken at 30 min (a–c) and 60 min (d–f). The reaction was stopped by the addition of one-fifth volume of 0.1 M EDTA (pH 8.0)–1% SDS. After addition of one-fifteenth of the original volume of 1.5 M NaCl–0.15 M sodium citrate, samples were treated twice with a chloroform–isoamylalcohol mixture (24:1, v/v). After the centrifugation (5,000 r.p.m., 30 min at  $4^\circ\text{C}$ ), the aqueous phase was dialysed against 50 mM Tris–HCl (pH 8.0)–50 mM EDTA–0.5 M NaCl. A 0.1 ml aliquot of each dialysed sample was treated with 0.24 M NaOH and applied to an alkaline sucrose density gradient (5–20%)<sup>16</sup>. a, d, Control, MR = 0; b, e, MR of diol-epoxide I is 1.4; c, f, MR of diol-epoxide I is 5.8.

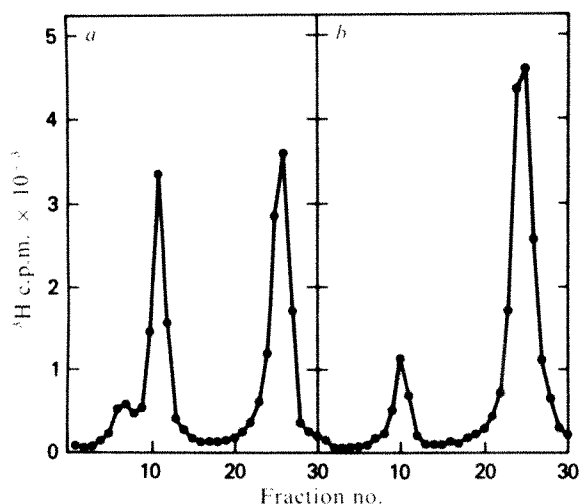
Figure 2 shows the alkaline sucrose density gradient profile of the product DNA. In normal conditions (MR = 0), the newly synthesised closed circular DNA banded near the bottom of the gradient (fractions 6–8). Unit sized single linear and single circular molecules banded at fractions 30 (15S). A peak which appeared at the top of the gradient (fractions 42–47) represents the 6S initiation fragments dissociated from the origin of pBR322 DNA replication<sup>11,13</sup>. The size classes of DNA synthesised during the first 30 min of incubation are identical and unrelated to the number of diol-epoxide I molecules bound to template DNA as observed. The only exception is that more 6S fragments accumulated in unmodified pBR322 DNA than in modified DNA, indicating that the formation of initial 6S fragments was slightly inhibited by diol-epoxide I binding.



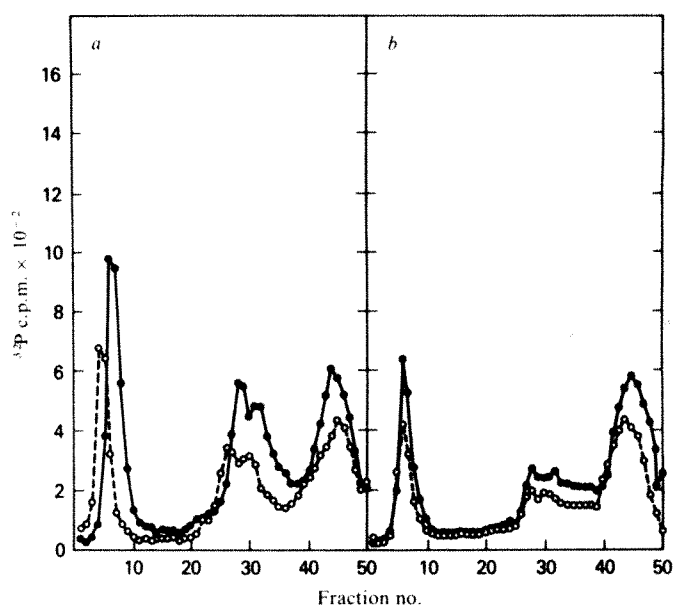
After 60 min of incubation, about four times more closed circular molecules had been synthesised from unmodified DNA than after 30 min (Fig. 2a, d). When diol-epoxide I was bound to DNA at MR = 1.4, the closed circular DNA synthesised was only doubled after an additional 30 min incubation (Fig. 2b, e). When MR = 5.8, the amount of closed circular DNA did not increase after an additional 30 min incubation (Fig. 2c, f). The number of unit-sized single-stranded linear or circular molecules (15S) markedly increased when intact DNA was subjected to replication as shown in Fig. 2a, d. This class of DNA also increased as much as that in the control experiment from 30–60 min when the DNA was bound to 1.4 molecules of diol-epoxide I (Fig. 2b, e). However, the peak at fractions 26–32 (15S) in Fig. 2e is markedly skewed towards the top of the gradient. Moreover, when the molar ratio was 5.8, there was no distinctive peak observed in the region of fractions 26–33 (Fig. 2f). In contrast to other classes of DNA, the relative proportion of 6S molecules synthesised did not change significantly in either control or modified DNA during an additional 30 min incubation. These results indicate that many intermediate sized newly synthesised DNA fragments with length less than the unit size of pBR322 DNA were accumulated when diol-epoxide I modified DNA was subjected to replication. The effect of covalently-bound diol-epoxide I is therefore to inhibit chain elongation with little effect on the initiation of DNA replication.

Note that pBR322 DNA, which carries the same base sequence as ColE1 DNA at the initiation site, consists of a high proportion of adenine and thymine residues<sup>10</sup>, which have significantly less chemical reactivity with diol-epoxide I than guanine residues<sup>5–7</sup>. Based on these findings and other studies, it may be reasonable to assume that the initiation site of DNA replication is free from the binding of diol-epoxide I and is therefore capable of generating a normal initiation of DNA synthesis when the molar ratio is small. The elongation of the deoxypolynucleotide chain was effectively blocked at the binding site of the template molecule. Hsu *et al.* have reported similar results regarding the inhibition of converting single-stranded  $\Phi$ X174 DNA to double-stranded circular DNA<sup>14</sup>.

Another question is whether the binding of diol-epoxide I completely blocks the elongation of the nascent DNA chain or whether the binding site can be excised and repaired, allowing



**Fig. 3** Analysis of heat-treated ColE1 DNA modified with diol-epoxide I in an alkaline sucrose density gradient centrifugation. Tritium-labelled ColE1 DNA was prepared after amplification with chloramphenicol as described previously<sup>16</sup>. The purified ColE1 DNA was modified with diol-epoxide I as described in the text. Unmodified ColE1 DNA (a) and modified ColE1 DNA (MR = 1.4) (b) were treated at 90 °C for 5 min in 5 mM Tris-HCl (pH 8.0)–5 mM EDTA–50 mM NaCl, and then the pH was adjusted to pH 12 with 4 M NaOH. Samples were analysed by the 5–20% alkaline sucrose density gradient as shown in the legend to Fig. 2.



**Fig. 4** Newly synthesised pBR322 DNA with (a) and without (b) diol-epoxide I (MR = 0 and 1.4 respectively) was analysed in an alkaline sucrose density gradient before and after heat-treatment. pBR322 DNA was subjected to the *in vitro* assay system for 60 min as described in the legend to Fig. 1. The product was analysed in the alkaline sucrose density gradient centrifugation with (dashed line) and without (solid line) heat-treatment as shown in the legend to Fig. 3.

the completion of replication. To investigate this, a method was devised to detect a small number of binding sites on closed circular DNA. About 50% of the closed circular DNA (MR = 0) was converted to single-stranded circular and linear forms in an alkaline sucrose density gradient after heat treatment of the DNA at 90 °C for 5 min (Fig. 3a). The conversion of 50% is a result of alkaline hydrolysis of the RNA segment which is presented in chloramphenicol-amplified ColE1 DNA<sup>15</sup>. The rate of conversion was clearly increased when diol-epoxide I was bound to ColE1 DNA (MR = 1.4), heated and analysed in an alkaline sucrose density gradient (Fig. 3b), indicating that the binding site itself is heat-alkali-labile. This provides a sensitive assay method for the detection of bound diol-epoxide I on DNA strands. When the method was applied to the DNA which had completed a round of replication (for method see legend to Fig. 2), the rate of conversion from closed circular to the other forms was no different for the control and modified (MR = 1.4) DNA (Fig. 4).

These results suggest that the parent strands no longer possess diol-epoxide binding sites which were present before replication and also rule out the possibility of the binding sites in the template being bypassed during replication. Excision of diol-epoxide-damaged DNA from the parental strand would best explain the reduction in the rate of DNA elongation described earlier.

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## Suppression of a yeast *amber* mutation in *Escherichia coli*

THE complementation of *Escherichia coli* auxotrophs by cloned eukaryotic genes<sup>1-4</sup> makes it possible to use standard bacterial genetic techniques to study these eukaryotic genes. Here, we describe the cloning of a *Saccharomyces cerevisiae* (yeast) gene with an *amber* suppressible allele. Reversion and suppression properties of this allele are examined in growing *E. coli* cells.

The yeast *his3* gene coding for imidazoleglycerolphosphate (IGP) dehydratase was cloned as a hybrid of bacteriophage  $\lambda$  which complemented *hisB* auxotrophs of *E. coli* lacking the analogous activity<sup>1</sup>. The wild type yeast *his3* gene is transcribed and translated in *E. coli* with high fidelity to produce an enzyme activity strongly resembling the activity found in yeast cells<sup>2</sup>. Complementation analysis of many derivatives containing *his3* sequences indicates that the *his3* structural gene is localised to a region of approximately 700 base pairs<sup>3</sup>. The length and location of this *E. coli* complementation unit is similar to the region homologous to the 650 base poly(A)-containing RNA species found in yeast cells (K. S., unpublished).

Genetic analysis in *E. coli* of the yeast *his3* gene depends on the availability of cloned derivatives which are nonfunctional. Mutant *his3* genes which are nonfunctional in *his3*<sup>-</sup> yeast cells are also nonfunctional when cloned and propagated in *E. coli*; that is, they do not complement *E. coli hisB* auxotrophs<sup>2</sup>. The order of two such *his3* lesions has been established by a three-factor cross of the bacteriophage  $\lambda$  hybrids containing the cloned mutant genes<sup>2</sup>. Non-complementing deletions of the yeast *his3* gene spontaneously generated during lytic growth of a bacteriophage  $\lambda his3$  hybrid have been isolated<sup>5</sup> by the method of Parkinson and Huskey<sup>6</sup>. The physical locations of the yeast lesions have been determined by deletion mapping in *E. coli*<sup>5</sup>. Deletion mutants which require transcriptional initiation from the  $\lambda$  promoter P<sub>L</sub> for *his3* expression have been used to define a yeast DNA sequence which functions in *E. coli* as a promoter<sup>7</sup>. Derivatives of a  $\lambda his3$  hybrid which overproduces IGP dehydratase activity in *E. coli* have also been isolated (M. Brennan and K. S., unpublished results).

The demonstration that cloned yeast genes can be reintroduced into yeast cells by transformation and expressed<sup>8</sup>

makes it possible to fuse the genetic systems of *E. coli* and *S. cerevisiae*. Recent findings indicate that DNA transformation of yeast can occur by at least three mechanisms (ref. 9 and J. B. Hick, A. Hinnen and G. R. F., unpublished results). Depending on the particular mechanism, transforming DNA can integrate into the yeast chromosomes by homologous recombination and/or replicate autonomously. Introduction of physically and genetically defined cloned *his3* derivatives into yeast cells should be an important tool in elucidating mechanisms of yeast regulation, DNA replication and recombination.

To aid such studies it would be useful to clone a conditionally lethal yeast mutation. Strain constructions would be significantly facilitated and interpretation of experimental results would be strengthened by the conditional nature of the lesion. To isolate a conditionally lethal lesion, 112 independently derived *his3* mutants of yeast<sup>10</sup> were screened for *amber* suppressibility by scoring for histidine prototrophy following mating with *his3*-532 SUP4-2. Three mutants (*his3*-14, *his3*-19 and *his3*-X5-21B) presumably containing internal UAG codons suppressible by tyrosine-inserting tRNAs<sup>11</sup> were isolated. DNA from strain *his3*-X5-21B was cloned in  $\lambda$ gt4 (ref. 12) by the *EcoRI*-DNA ligase method<sup>2</sup>. From this pool of  $\lambda$ gt4 hybrids, a phage containing *his3* sequences was isolated by the plaque filter hybridisation method of Benton and Davis<sup>13</sup>. The 10.1 kb (kilobase pair) *EcoRI* fragment in this phage, Sc2693, is physically indistinguishable from the analogous fragment (Sc2601) which complements *E. coli hisB* mutations.  $\lambda$ gt4-Sc2693 does not complement *hisB*463; therefore, the phage contains a cloned yeast *his3* gene with an *amber* lesion. The *his3* gene is located internally within a 1.7 kb *Bam*HI fragment of Sc2601 DNA<sup>5</sup>. The equivalent *Bam*HI DNA fragments of  $\lambda$  hybrids containing *his3* mutant genes were cloned in the yeast vectors YIp5 and YRp7 (ref. 9). The sources of these cloned mutant genes were  $\lambda$ gt1-Sc2612 (*his3*-38),  $\lambda$ gt6-Sc2679 (*his3*-532) (ref. 2), and  $\lambda$ gt4-Sc2693 (*his3*-X5-21B).

All mutant *his3* genes cloned in these yeast vectors were introduced into the *E. coli* strain *hisB*463 by selection for the plasmid-coded gene for ampicillin resistance. The spontaneous reversion rates in *E. coli* of these yeast lesions were measured and compared to the spontaneous reversion rates in yeast (Table 1). The comparison is complicated by the fact that in these *E. coli* cells, the *his3* genes are presumably present in about 20 copies, and that the expression is only about four times above the single copy level<sup>7</sup>. The reversion rates are measured as His<sup>+</sup> colonies obtained per cell. It is unclear how these numbers are related to number of reversion events per DNA molecule. Multiple copies of *his3*-38 in *hisB*463 cells allow some growth in the absence of histidine. The reversion rate of this lesion in *E. coli* as determined from plasmid hybrids ( $2 \times 10^{-8}$ ) agrees well with the previous results obtained with phage hybrids<sup>2</sup>. The reversion rate of this lesion in yeast is significantly lower ( $<10^{-9}$ ). *his3*-532 is a very stable lesion in both organisms, though revertants are detected in *E. coli* but not in yeast *his3*-X5-21B reverts in *E. coli* at a significantly lower rate than it reverts in yeast. These results indicate that spontaneous reversion of a given yeast lesion occurs at different rates in yeast and in *E. coli*.

Table 1 Suppression and reversion characteristics of yeast *his3* lesions

<i>his3</i> lesion	$\lambda$ Hybrid	Plasmid hybrid	<i>hisB</i> (supF)	<i>his</i> <sup>+</sup> colonies <i>hisB</i>	Yeast
<i>his3</i> -38	$\lambda$ gt1-Sc2612	YIp5-Sc2719	$2 \times 10^{-8}$	$3 \times 10^{-8}$	$<10^{-9}$
		YRp7-Sc2719	$1 \times 10^{-8}$	$1 \times 10^{-8}$	
<i>his3</i> -532	$\lambda$ gt6-Sc2679	YIp5-Sc2720	$2 \times 10^{-10}$	$1 \times 10^{-10}$	$<10^{-10}$
		YIp5-Sc2721	1	$3 \times 10^{-9}$	
<i>his3</i> -X5-21B	$\lambda$ gt4-Sc2693	YRp7-Sc2721	1	$1 \times 10^{-9}$	$2 \times 10^{-7}$

As *his3*-X5-21B is suppressed in yeast by a tyrosine-inserting tRNA, suppression in *E. coli* was examined with an analogous tRNA species (*supF*). *hisB463* cells containing the cloned yeast *his3* lesions were lysogenised by a phage ( $\phi 80$  *supF*) containing the *E. coli* gene coding for a tRNA species capable of suppressing amber (UAG) codons<sup>14</sup>. Introduction of the *supF* allele into these *hisB463* derivatives was determined by their ability to plate  $\lambda$ cI857Sam7. *hisB463* cells containing both the *his3*-X5-21B and the *supF* alleles grow in the absence of histidine (Table 1); that is, the *his3* lesion is suppressed by *supF*. This result is not due to an artefact of selection, as both the *his3*-X5-21B and the *supF* alleles were introduced into the *hisB463* host by non-selective means. Furthermore, it confirms the amber suppressibility of the *his3*-X5-21B allele. It is now possible to introduce DNA containing this conditionally lethal yeast gene back into yeast and *E. coli*.

These results indicate that an *E. coli* amber-suppressing tRNA species suppresses a yeast amber lesion in growing *E. coli* cells. It has been previously established that purified yeast suppressor tRNAs, when present in heterologous, eukaryotic, *in vitro* translation systems, suppress prokaryotic (Q $\beta$ ) amber mutations<sup>15,16</sup>. Taken together, these *in vivo* and *in vitro* experiments provide evidence that translational termination and tRNA suppression of nonsense codons are processes which are conserved between prokaryotic and eukaryotic organisms.

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## Genotype control of the dystrophin muscularis gene in mice

MUTANTS of the dystrophin muscularis gene (*dy*) in mouse suffer from progressive skeletal muscle loss, together with inadequate repair or regeneration<sup>6</sup>. In 1974 I reported a distinctive phenotypic difference between two dystrophic mutant alleles with respect to myogenesis *in vitro*<sup>1</sup>. Homozygous *dy*<sup>21</sup> mice gave cultures in which there was apparently normal myogenesis, whereas mice homozygous for *dy* gave cultures which contained large numbers of grouped uninucleate myoblasts

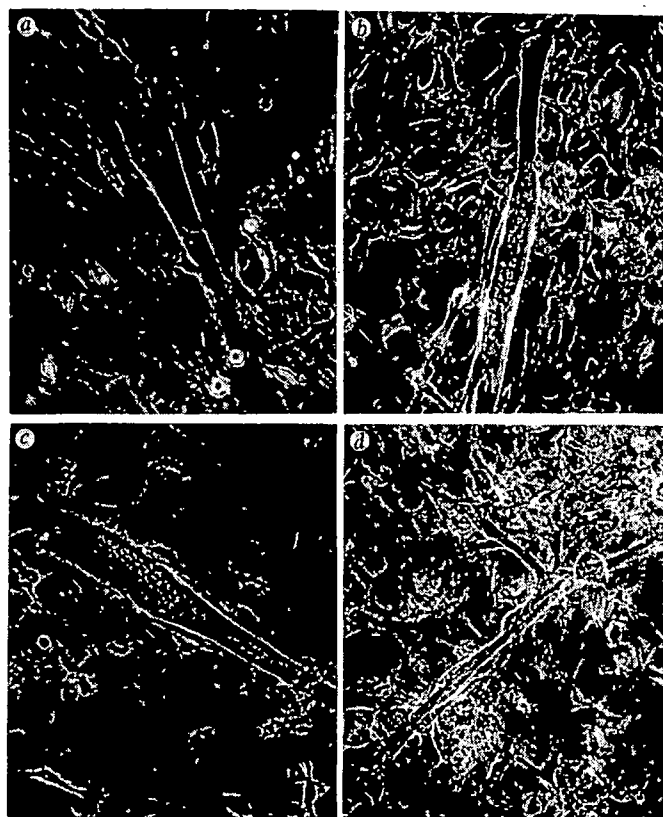


Fig. 1 Unstained living cultures photographed with phase contrast-optics. The structures are typical and the illustrations representative of the experimental results. a, C57 +/+, normal multinucleate myotube; b, C57 *dy*<sup>21</sup>/*dy*<sup>21</sup>, large syncytial myotube; c, C57 *dy*/*dy*, a definite multinucleate myotube; d, 129 *dy*/*dy*, characteristic pseudostraps with discrete uninucleate cells. All photographs were taken at 4 d *in vitro* in identical conditions.

(pseudostraps) in place of multinucleate syncytial myotubes<sup>2</sup>. MacPike, however, showed<sup>3</sup> that muscular dystrophies caused by these two alleles carried in the same genetic background (C57BL/6J) were similar when assessed histologically. I have, therefore, investigated how the process of myogenesis *in vitro* (which can be considered to be another independent function of the muscle) is affected not only by the two allelic mutants but also by the genetic background. The *dy* dystrophy was known to be histologically similar in either the C57BL/6J or 129/ReJ background<sup>3</sup> but surprisingly C57BL/6J *dy*/*dy* cultures gave normal myogenesis while 129/ReJ *dy*/*dy* cultures again produced pseudostraps.

The mice used in this series of experiments were C57BL/6J *dy*<sup>21</sup>/*dy*<sup>21</sup>, C57BL/6J *dy*/*dy*, C57BL/6J +/+ and 129/ReJ *dy*/*dy* at 2, 3, 4 and 5 months of age. Cultures were obtained from 4 day crush lesions as described previously<sup>1</sup>. Daily examination for a period of 8 days was carried out blind; 48 cultures of each genotype were assessed. As before, the pattern of growth during the first 3 days was one of proliferation and explant spreading. Myotubes, increasing in size and number, developed by days 4 to 8 in all three C57BL/6J genotypes including the *dy* mutant (Fig. 1a, b, c). The 129/ReJ *dy*/*dy* showed without exception the characteristic pseudostraps (Fig. 1d) and the occasional small myotube during the first 3 days.

*In vitro* the 129/ReJ *dy* muscle attempts to form myotubes but does not succeed<sup>1</sup>. However, the same muscle in a more complex organotypic culture of fetal spinal cord and muscle exhibits some regeneration<sup>4</sup> although it is much less than normal muscle in the same conditions. Furthermore, the *dy* muscle cells cultured as a low density monolayer<sup>5</sup> produce apparently normal myogenic colonies which contain well differentiated muscle fibres. Clearly, myogenesis from dystrophic muscle is extremely susceptible to environmental conditions. But it is also



clear from the experiments reported here that there is a positive indication of a genotypic control over dystrophic myogenesis.

The consequence of these findings of the interaction between the gene at the *dy* locus and any other genes of a particular background is clearly important with regard to the interpretation of experiments, past and future.

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## Sensitivity of low molecular weight RNA synthesis to UV radiation

RNA SPECIES C and D (nomenclature of Weinberg and Penman<sup>1</sup>) are among the most abundant of the long-lived<sup>2</sup>, homodisperse, low molecular weight nuclear RNAs<sup>3</sup>, and are present in all vertebrates tested<sup>4</sup>, but their function is unknown. They have several interesting characteristics: they seem to be transcribed from multiple-copy genes ( $10^2$ - $10^3$  genes per genome)<sup>5,6</sup>, to pass through the cytoplasm for a few minutes shortly after their transcription<sup>3,7-10</sup>, to have 5'-end caps that are very similar to those of eukaryotic mRNAs<sup>11,12</sup>, and to be present in heterogeneous nuclear RNA-protein particles<sup>13,14</sup>. Here, we show that C and D RNA syntheses are unexpectedly sensitive to UV radiation in HeLa cells. The effect of UV radiation on RNA synthesis has been used to study the organisation of transcription units<sup>15</sup>. UV irradiation, causing random formation of pyrimidine dimers in DNA, results in premature termination of transcription. The probability of causing a UV lesion within a transcription unit is directly proportional to its length, making it possible to measure the distance between a gene and its promoter. The present UV sensitivity data are compatible with the transcription units for the low molecular weight (less than 200-nucleotide long<sup>1,11,12</sup>) RNA species C and D being as long as 5 kilobases.

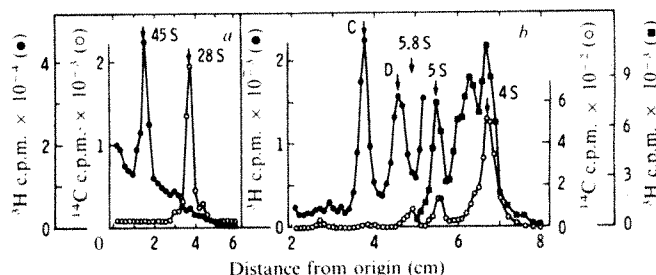
In this study, RNA was extracted from whole cells and analysed by polyacrylamide gel electrophoresis. Figure 1 shows an example of the routine assay used. Recovery variations were corrected by the <sup>14</sup>C-labelled RNA bands, as the cells had been incubated with <sup>14</sup>C-uridine for 1 d before the experiment. The briefly labelled RNA migrating between 5S and 4S RNA seems to be mainly 4S RNA precursors<sup>16,17</sup>, and so it was included in the estimations of newly made 4S RNA. If any RNA fragments were generated by UV effects on large transcription units<sup>18</sup>, they might have raised the radioactivity background in the gel electrophoresis pattern. This would not have affected our estimates, because only the counts of a given peak above this background were computed. These results would not have been affected by any possible effect of UV radiation on the cytoplasmic-nuclear maturation of C and D RNA, as whole cell RNA was assayed.

Figure 2a shows the sensitivity of the synthesis of 45S rRNA to UV radiation. It is assumed that brief labelling of RNA (20 min with <sup>3</sup>H-uridine) approximates RNA synthesis, as precursor pools do not seem to be affected by UV radiation<sup>18</sup>. The UV dose that resulted in a 37% level of residual synthesis ( $D_{37}$ ) was near 8 s when the incident dose rate was  $34 \text{ erg mm}^{-2} \text{ s}^{-1}$ .

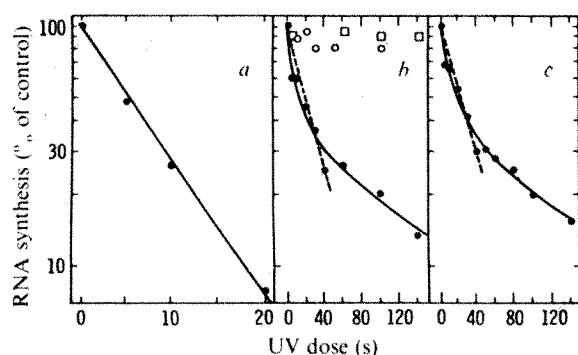
The resulting product is almost identical to the  $D_{37}$  incident dose of  $270 \text{ erg mm}^{-2}$  reported for mouse L cell 45S rRNA synthesis<sup>19</sup>. Figure 2b and c shows that the synthesis of C and D RNA was inhibited, whereas the synthesis of 4S and 5S RNA was essentially unaffected at this dose range of UV radiation, as had been shown by Goldberg *et al.*<sup>20</sup>. In addition, the inactivation curve for both C and D RNA synthesis deviated from exponential kinetics (Fig. 2b, c). The  $D_{37}$  value for both C and D RNA synthesis, calculated by first order approximation of the initial slope (broken straight lines in Fig. 2b, c), was nearly 30 s. At an incident dose rate of  $34 \text{ erg mm}^{-2} \text{ s}^{-1}$ , the  $D_{37}$  incident dose for C and D RNA synthesis is then about  $1 \times 10^3 \text{ erg mm}^{-2}$ . The  $D_{37}$  incident dose for 18S rRNA synthesis is  $880 \text{ erg mm}^{-2}$  (ref. 19), whereas the distal end of the 18S rRNA gene is located about 5 kilobases from its promoter, as determined by electron microscopic<sup>21</sup> and UV transcription mapping<sup>19</sup>.

Two models could account for our results. Based on the  $D_{37}$  value, the C (or D) RNA sequences would be derived from 4-5-kilobase-long transcription units, and according to the deviation from exponential kinetics, several C (or D) RNA genes would be tandemly arranged behind a common promoter<sup>19</sup>. It can then be estimated that at least seven gene copies would exist per transcription unit<sup>19</sup>. Alternatively, the present data are compatible with the synthesis of a population of original transcripts which are heterogeneous in size, vary in length between approximately 0.5 and 5 kilobases, and which have a C (or D) RNA sequence at or near the 3'-terminus of each polynucleotide. Either model is unexpected because the known precursors to C and D RNA are about 0.2 kilobases long<sup>9,22</sup>. The precursors of C and D RNA, which are only slightly larger than the mature species, can be detected after at least 4 min of labelling<sup>7</sup>. It therefore follows that the proposed large original transcripts would be cleaved shortly after or during transcription.

There is no detectable repair of the lesions responsible for premature termination of transcription of rRNA<sup>19</sup>, histone<sup>23</sup> and adenovirus type 2 (ref. 24) genes, for at least 60-90 min after UV irradiation of mammalian cells. Therefore, it seems unlikely that repair of UV damage would significantly affect our results in the 30 min after UV irradiation in the present experiments. In any case, the unusual feature of the present data is the high sensitivity to UV radiation of C and D RNA synthesis, and fast repair would have only masked some of this sensitivity. It could be argued that the proposed interpretations of the data might be erroneous because UV radiation may affect transcription by various eukaryotic RNA polymerases differently. It has already been shown that UV transcription mapping works well with transcription by RNA polymerases class I and II (refs 19, 20, 25, 26), and one of these two types of polymerase is suspected to be involved in the synthesis of C and D RNA<sup>27,28</sup>. It



**Fig. 1** Polyacrylamide gel electrophoresis patterns of large (a) and small (b) RNAs from whole cells. HeLa S3 cells were labelled for 1 d with <sup>14</sup>C-uridine<sup>7</sup>, and then exposed to UV irradiation<sup>2</sup>, placed at 15 cm from a GE G2578 lamp (incident dose rate of  $34 \text{ erg mm}^{-2} \text{ s}^{-1}$ ). The cells were then spun down, and resuspended at  $2 \times 10^6$  cells  $\text{ml}^{-1}$  in medium and serum; after 10 min at  $37^\circ \text{C}$ , [<sup>3</sup>H-5]uridine ( $20 \mu\text{Ci ml}^{-1}$ ,  $26 \text{ Ci mmol}^{-1}$ ) was added, and the cells were finally collected after labelling for 20 min at  $37^\circ \text{C}$ . RNA was extracted from whole cells with phenol and SDS at  $55^\circ \text{C}$ , and analysed by 2.4% (a) and 10% (b) polyacrylamide gel electrophoresis as described elsewhere<sup>29</sup>. ■, <sup>3</sup>H (contracted scale); ●, <sup>3</sup>H (expanded scale); ○, <sup>14</sup>C.



**Fig. 2** Effect of UV radiation on labelling of several RNAs in HeLa cells. *a*, 4S rRNA; *b*, C RNA (●), 4S RNA (□) and 5S RNA (○); *c*, D RNA. The broken lines represent pseudo-first-order approximations to the experimental data. All conditions were as indicated in the legend to Fig. 1.

seems unlikely that C and D RNA could be synthesised by RNA polymerase class III, because in the presence of  $\alpha$ -amanitin, when 5S and 4S RNA synthesis is essentially unaffected, the synthesis of C and D RNA is markedly inhibited<sup>28</sup>.

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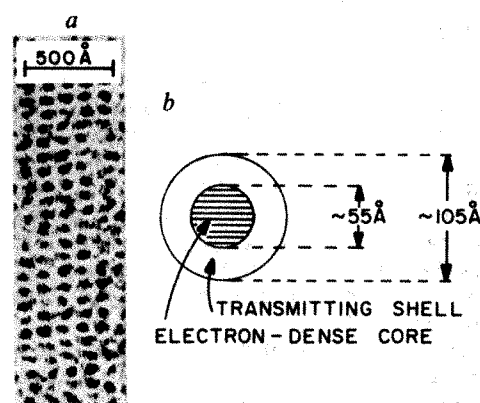
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## ***Azotobacter* cytochrome *b*<sub>557.5</sub> is a bacterioferritin**

FERRITIN is the presumed iron storage protein of mammalian<sup>1</sup>, plant<sup>2</sup> and certain fungal<sup>3</sup> systems. The nitrogen-fixing bacterium *Azotobacter vinelandii*, produces a *b*-type cytochrome containing large amounts of non-haem iron and no labile sulphide, as first shown by Bulen *et al.*<sup>4</sup>. Here we present

evidence which reveals this protein to be a ferritin-like species, thus constituting the first authenticated occurrence of ferritin in a bacterium. The interesting redox properties of this molecule, bacterioferritin-cytochrome, are presented and discussed.

The cytochrome *b*<sub>557.5</sub> was prepared by the method of Bulen *et al.*<sup>4</sup> from N<sub>2</sub>-fixing *A. vinelandii* cells collected after they had entered the stationary phase. The purification method for this acidic protein involves heat treatment, protamine sulphate precipitation, dissolution by cellulose phosphate treatment and repeated crystallisation by the addition of Mg<sup>2+</sup>. The preparation is homogeneous on polyacrylamide gel electrophoresis, and SDS gel electrophoresis reveals a single subunit. The Fe content of 2.8–3.5  $\mu$ mol Fe per mg protein requires about 100 iron atoms per haem molecule in the preparation. As the iron constitutes 13–20% of the weight of the protein, we investigated the possible relationship of this protein to ferritin.



**Fig. 1** *a*, Small portion of a transmission electron micrograph of a crystalline sample of bacterioferritin-cytochrome of *A. vinelandii*. Sample preparation involved fixation with glutaraldehyde, dehydration with ethanol and embedment in Spurr's low viscosity embedding medium. No stain was used. *b*, Schematic idealisation of the dense inner region and 'transparent' outer shell of the bacterioferritin-cytochrome as revealed in the electron microscope. The regions are assigned, respectively, to the Fe core and protein shell.

Table 1 compares various analytical properties of this molecule with those of horse spleen ferritin<sup>5–8</sup>. SDS gel electrophoresis reveals a single subunit of molecular weight (MW) 17,000, slightly smaller than that of mammalian ferritin<sup>6</sup>. Amino acid analysis shows the expected dominance of acidic amino acids and yields a nearest integer MW of 17,185. Although the similarity to ferritin is already apparent, the most striking indication of the ferritin nature of the protein comes from electron microscopy of the unstained crystalline preparation. The portion of an electron micrograph shown in Fig. 1 reveals the presence of electron-dense cores of  $\sim 55$  Å diameter within approximately spherical electron transmitting shells of diameter  $\sim 105$  Å. The outer shells are presumed to be of protein, and the inner core must contain an iron compound (see below). These dimensions are virtually identical to those found by electron microscopy for mammalian ferritin<sup>9</sup>. Similarly, the apparent variable occupancy of the inner cores parallels that found in ferritin<sup>1–3,9</sup>.

Crystalline samples of the molecule have been studied by Mössbauer spectroscopy of <sup>57</sup>Fe at natural abundance by Lang and Spartalian (in preparation), and reveal a quadrupole-split doublet characteristic of Fe<sup>3+</sup> at 77 K. As the temperature is lowered to 1.5 K, a six-line magnetically split multiplet appears. The limiting high and low temperature spectra are virtually identical to those found previously<sup>10–12</sup> for horse ferritin. Magnetic susceptibility of the *Azotobacter* protein at 34 °C by the Evans NMR method<sup>13</sup> gives a magnetic moment,  $\mu_{\text{eff}} = 3.7$  Bohr magnetons per Fe atom. The same experiment for horse spleen ferritin (Sigma) also yields 3.7 BM per Fe atom, in

agreement with previous work<sup>14,15</sup>. The Mössbauer and susceptibility experiments together establish the weakly coupled high-spin ferric nature of the bulk iron.

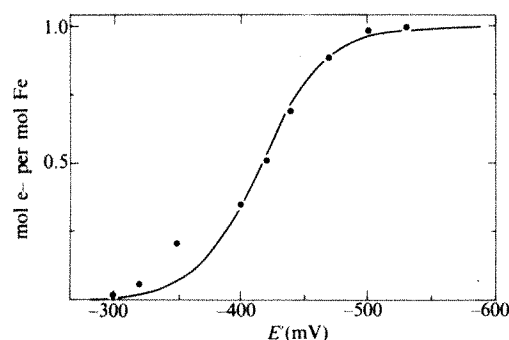
The combined information from the mode of preparation and crystallisation, the high Fe content, the electron microscopy, the amino acid composition, the subunit MW, the Mössbauer spectra and magnetic susceptibility, lead us to conclude that this protein represents the first example of a bacterioferritin.

It was the presence of haem that led to the original purification of the protein<sup>4</sup>. The electronic spectrum of the oxidised form has a Soret band at 417 nm as the only distinct peak in the visible region<sup>4</sup>. On reduction with  $S_2O_4^{2-}$ /methyl viologen, we find a clean reduced cytochrome spectrum with  $\alpha$ ,  $\beta$  and Soret bands at 557.5, 527 and 425 nm, respectively.

To show the intimate association of haem with this bacterioferritin, we note first that extensive dialysis and repeated recrystallisation can be accomplished without change in composition. Further, polyacrylamide gel electrophoresis, isoelectric focusing and gel-filtration chromatography each reveal a homogeneous preparation and establish the inseparability of haem and non-haem iron components of the protein. Although the haem has been identified as protoporphyrin IX (ref. 4), quantitative determination by the pyridine haemochromogen method is precluded by variable absorption (or occlusion) of haem by the precipitated non-haem iron cores. Haem was therefore determined by treatment with formic acid, which unfolds the protein, solubilises the non-haem iron and presumably stabilises the ferrihaem as the formic acid complex. With protein determined by the Lowry procedure<sup>16</sup> and haem absorption in formic acid standardised with haemin chloride or haemoglobin, we initially find an average of 37,000 daltons per haem. We note that one haem for two subunits requires 34,000 daltons per haem. In addition to the apparent inseparability of the haem and non-haem iron and the roughly constant haem/protein ratio, the redox properties of the protein show an intimate relationship of haem and non-haem iron.

Coulometric titrations<sup>17</sup> (Fig. 2) reveal a reversible Nernst plot (for  $n = 1$ ) with a potential of  $-416$  mV at pH 7 using methyl viologen as the mediator. Coulometry shows that for most samples one electron is taken up for each iron atom present in the molecule. Spectroscopy reveals that both the haem and the large excess of non-haem iron are reduced in this process. Potentiometric titration of the protein, carried out by monitoring the appearance of the  $\alpha$  band of the reduced haem, reveals that the haem undergoes reduction at a potential only 30 mV more negative than that of the bulk iron. Thus, both the haem and non-haem iron are reducible at similar extremely low redox potentials. These potentials are significantly more negative than those required to reduce horse spleen ferritin.

We have preliminarily explored the fate of the reduced *Azotobacter* protein. Our initial experiments show that the coulometrically fully reduced protein can be passed through an



**Fig. 2** Coulometric titration of bacterioferritin-cytochrome of *A. vinelandii* at 25 °C and pH 7 versus the normal hydrogen electrode using methyl viologen as mediator by the method described in ref. 12. The points are experimental values and the curve is calculated from the Nernst equation for  $n = 1$ ,  $E' = -416$  mV.

**Table 1** Comparison of *Azotobacter* protein with horse spleen ferritin

Aminoacid	<i>Azotobacter</i> bacterioferritin-cytochrome	Horse spleen ferritin
Cys	2.3	2.9
Asx	17.2	17.3
Thr	2.2	5.5
Ser	5.3	9.0
Glx	26.8	23.9
Pro	2.3	2.8
Gly	9.9	9.9
Ala	8.0	14.0
Val	1.9	6.9
Met	4.4	2.8
Ile	12.2	3.5
Leu	25.7	25.0
Tyr	5.7	5.0
Phe	2.1	7.3
Trp	2.4	2.1
His	6.9	5.8
Lys	11.7	8.7
Arg	3.1	9.5
Subunit MW (SDS gel electrophoresis)	17,000*	18,500

The analysis of *Azotobacter* bacterioferritin-cytochrome was carried out on protein samples without Fe removal. Similar analyses to those shown here on this protein by D. Jacobs and the late W. A. Bulen (D. Jacobs, personal communication) give values which differ from those reported here by less than a single residue for each amino acid. Data for horse spleen ferritin are from ref. 6.

\*Obtained by the procedure of Weber, Pringle and Osborn<sup>30</sup> using, as calibrants, aldolase, pepsin, chymotrypsin, trypsin, ferritin, myoglobin, haemoglobin, lysozyme and cytochrome *c*.

anaerobic Biogel P-2 column such that >90% of the (ferrous) iron is maintained in the reduced protein, which elutes with the solvent front. Separate experiments demonstrate that the column easily separates reduced ferritin-cytochrome from added ferrous ions. The eluted fully reduced ferritin-cytochrome can then be rapidly reoxidised by air to a species which is spectroscopically and analytically indistinguishable from the original oxidised material, with the exception of a small loss of Fe. The *Azotobacter* protein seems to hold its iron longer in the reduced state than does mammalian ferritin.

We conclude that *A. vinelandii* produces a protein with dual bacterioferritin-cytochrome character. If the molecule resembles ferritin in quaternary structure (as seems likely from the electron microscopy), then its 24 subunits, ~12 haems and average of 1,600 Fe atoms in the oxide-hydroxide-phosphate core give a material of MW 660,000 capable of taking up or delivering up to 1,600 electrons. It differs from mammalian ferritin in that it contains haem, has a lower redox potential and holds its iron more tenaciously in the  $Fe^{2+}$  state. We can only speculate on the physiological function and biological ubiquity of this bacterioferritin-cytochrome.

The high iron content makes this protein a prime candidate for an iron storage protein of *A. vinelandii*, much as ferritins have this function in mammalian, plant and fungal systems<sup>1-3</sup>. If Fe storage is indeed its physiological role, then, as has been postulated in mammalian systems<sup>18-21</sup>, iron mobilisation may be effected by the reduction of  $Fe^{3+}$  to the more labile and more soluble  $Fe^{2+}$  state. Perhaps the presence of the haem in the same molecule facilitates the reduction of the internal ferritin Fe. Why, then, is such a low potential required to mobilise the Fe? An intriguing hypothesis is that the Fe in the ferritin-cytochrome is a specific iron-storage depot for nitrogenase and its low redox potential ensures that only when the local redox potential in the cell approaches the negative values required for nitrogenase turnover (~-430 mV)<sup>22</sup> will the iron be mobilised from the ferritin-cytochrome.

An alternative or possibly additional role of the bacterioferritin-cytochrome involves its functioning as an electron storage



protein. The ability of this single molecule to take up hundreds of electrons at a low redox potential suggests that in *Azotobacter* this protein may function to supply low potential redox equivalents for use in respiration, biosynthesis or nitrogen fixation.

Many bacteria<sup>23</sup>, including *Azotobacter*<sup>24</sup>, excrete siderophores in conditions of Fe deficiency. It seems likely, therefore, that in conditions of iron sufficiency, species other than *A. vinelandii* will also have developed the ability to make bacterioferritin. Furthermore, some of these bacterioferritins could contain haem as does the *Azotobacter* protein. Spectroscopically, the haem absorptions found in the *A. vinelandii* protein are extremely similar to those found in cytochromes *b*<sub>1</sub> (often of unknown physiological function) from various bacterial sources<sup>25</sup>. Although none of the other cytochromes *b*<sub>1</sub> have been reported as containing non-haem iron, these do share with the *A. vinelandii* ferritin-cytochrome a relatively low redox potential and a tendency to be present in high MW aggregates. As cytochrome *b*<sub>1</sub> purification procedures often involve the use of detergents (which would cause loss of Fe<sup>3+</sup> core and leave apoferritin), the possibility remains that these are the bacterioferritin-cytochrome analogues of apoferritin.

In mammalian Fe metabolism, a close relationship between ferritin and haem metabolism has been postulated in mitochondrial particles, and evidence for direct involvement of ferritin in haem biosynthesis has been considered<sup>26-29</sup>. Further studies of bacterioferritins and/or bacterioferritin-cytochromes may help to establish evolutionary links which could improve our comprehension of both microbial and mammalian iron metabolism.

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## Structure of the iron-sulphur clusters in *Azotobacter* ferredoxin at 4.0 Å resolution

A FERREDOXIN-LIKE protein from *Azotobacter vinelandii* having 6-7 Fe and 6-7 S<sup>2-</sup> per mol and a molecular weight of 14,000 has been described by Shethna<sup>1</sup> and Yoch *et al.*<sup>2</sup>. Its biochemical properties<sup>3</sup>, electron paramagnetic resonance and redox behaviour<sup>4,5</sup>, and primary sequence<sup>6</sup> have also been studied. This work has established that there are two Fe-S centres separated by 0.744 V in reduction potential, one behaving like the [Fe<sub>4</sub>S<sub>4</sub>S<sub>4</sub><sup>2+</sup>] cluster in high-potential iron proteins, the other displaying novel characteristics. The distribution of cysteines in the N-terminal sequence is distinctly non-homologous with clostridial ferredoxins. Extrusion of the Fe-S cores by thiol displacement produces unique species, suggesting the presence of a new chromophore structure<sup>7</sup>. I report here crystallographic studies of the protein in a tetragonal crystal form which have led to an electron density map at 4.0 Å resolution. This map reveals two Fe-S clusters of clearly different size and shape. It has not been previously shown that this Fe-S protein, or any other, actually contains clusters of differing structure.

The protein was isolated by the method of Shethna<sup>1</sup> ( $A_{280}/A_{400} = 1.7-1.8$ ) and crystallised as square bipyramidal prisms<sup>8</sup>. The crystals are tetragonal, space group P4<sub>3</sub>2<sub>1</sub>2 with  $a = 55.24$  Å,  $c = 95.05$  Å, and one molecule per asymmetric unit. Diffractometer data have been collected to 4.0 Å resolution from single crystals of the native and an isomorphous derivative prepared with K<sub>2</sub>PtCl<sub>6</sub>. Data were collected at 10 °C using CuKα radiation and an  $\omega$  step-scan procedure. Bijvoet pairs were measured at  $\pm 2\theta$  in blocks of 10 reflections. After 6 d of irradiation, decay at 4.0 Å was 20%.

Table 1 Single isomorphous replacement phase sets

Phase Set	Space group	Pt			<i>E</i>	<i>R</i> <sub>c</sub>	$\langle m \rangle$
		<i>x</i>	<i>y</i>	<i>z</i>			
1	P4 <sub>1</sub> 2 <sub>1</sub> 2	0.48	0.13	0.19	33.3	0.478	0.49
2	P4 <sub>1</sub> 2 <sub>1</sub> 2	-0.48	-0.13	-0.19	38.9	0.552	0.47
3	P4 <sub>3</sub> 2 <sub>1</sub> 2	0.48	0.13	0.19	39.4	0.547	0.47
4	P4 <sub>3</sub> 2 <sub>1</sub> 2	-0.48	-0.13	-0.19	33.1	0.501	0.49

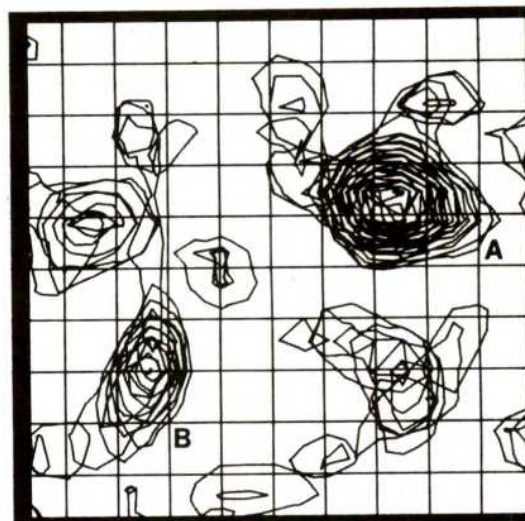
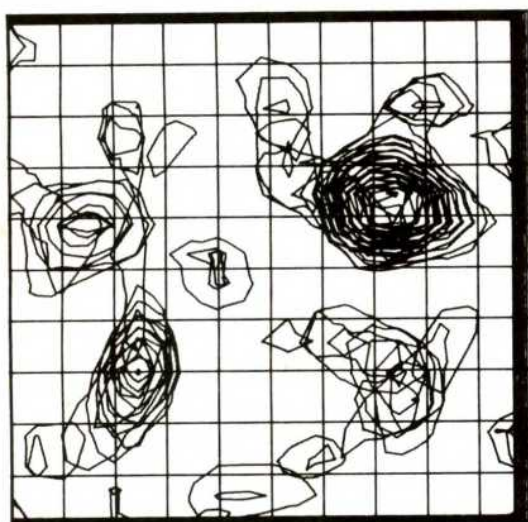
$$E = \frac{\sum_n ||F_{PH}| - |F_P + f_H||}{n}$$

where  $F_P$ ,  $F_{PH}$  are observed structure amplitudes for native and derivative, respectively, and  $f_H$  is calculated heavy atom structure amplitude, and  $n = 1,416$  reflections.

$$R_c = \frac{\sum_{hkl} ||F_{PH}| - |F_P + f_H||}{\sum_{hkl} ||F_{PH}| - |F_P||}$$

for 475 centric reflections (0*kl*, *hk*0, *hhl*).

$\langle m \rangle$ , Mean figure of merit.



**Fig. 1** Electron density map of *Azotobacter ferredoxin*. Fe-S clusters are marked A, B. Seven sections on  $x$ , 0.23–0.35;  $y$  axis vertical, 0.18–0.52;  $z$  axis horizontal, 0.23–0.43. Grid spacing  $\sim 1.9$  Å. Contours start at 18% of highest density (cluster A) with intervals of 10%.

The isomorphous difference Patterson map for the derivative showed six peaks (average peak to background ratio 3:1) consistent with a single major Pt site. Patterson maps using data from three additional Pt-soaked crystals in trial experiments were similar. The correct hand of both the heavy atom position and the space group was determined unambiguously by using the anomalous scattering from iron. Four protein phase sets were derived using the Pt data without anomalous dispersion (Table 1). In each case Bijvoet difference<sup>9</sup> and 'best' Fourier maps were computed using the native data. Phase set 1 yielded two large positive peaks in the 'best' Fourier at the Fe-S cluster sites, but large negative peaks at these sites in the Bijvoet difference map. Phase sets 2 and 3 produced uninterpretable maps. With phase set 4 both Fourier maps contained large positive peaks at the Fe-S sites at positions related by  $1/2-z$  from the set 1 maps, establishing this as the correct enantiomer. Subsequently, the heavy atom parameters and protein phases were refined in  $P4_32_12$  with Pt anomalous data included (Table 2). Difference Fourier maps revealed no secondary Pt binding sites. A Bijvoet difference Fourier with Pt anomalous differences again showed positive peaks at the Fe-S sites as well as a peak at the Pt site, the highest in the map and more than twice as high as the Fe-S peaks.

The electron density map in the region of the Fe-S clusters is shown in Fig. 1. The clusters are separated by 11 Å and occur at 0.26, 0.39, 0.38 (A) and 0.30, 0.28, 0.28 (B). Several stretchings of the polypeptide chain can be traced in the native map, including a right-handed helical segment. A clear solvent boundary delineates a molecule  $30 \times 25 \times 35$  Å in size. The map is featureless at the Pt site, which occurs on the molecular surface 19 Å and 13 Å from clusters A and B, respectively.

**Table 2** Refinement parameters for  $K_2PtCl_4$  derivative to 4.0 Å

$A^*$	62.88	$f_H$	126.8
$x$	0.4816	$E$	45.7
$y$	0.1352	$R_c$	0.478
$z$	0.3155	$\langle m \rangle$	0.66
$B$	28.00 Å <sup>2</sup>	$n$	1416

\* Occupancy on scale of  $f_H$ ,  $E$  (see Table 1 for definitions).

The most striking feature of the electron density is the differing shape and weight of the Fe-S peaks. Cluster A is essentially a 6 Å diameter sphere, consistent with the expected presence of a  $[Fe_4S_4S_4^{Cys}]$  cluster(s), like those seen in *Pepto-*

*coccus aerogenes ferredoxin*<sup>10</sup> and high-potential iron protein<sup>11</sup>. Cluster B, however, is significantly flattened and oblate in shape, being at most 4.5 Å thick. The maximum density at its centre is 75% that of cluster A. At 5.0 Å resolution, this cluster has a similar appearance but is only 55% the height of cluster A. Density maps calculated with centric or acentric phases only also give the same image of the Fe-S clusters. Thus, the electron density cannot be interpreted in terms of a second  $Fe_4S_4$  cube. Although it is not possible to distinguish unequivocally between other possible structures at this resolution, the density for the smaller cluster does suggest a two-iron centre, in analogy with model compounds of the type  $Fe_2S_2(RS)_4$  (ref. 12). This interpretation is supported by the fact that the protein chains attach to cluster A from several directions (two shown in Fig. 1), whereas cluster B is attached only at opposite ends.

A 4Fe-2Fe model agrees with the original Fe and  $S^{2-}$  assay and is consistent with the sequence. As the crystals diffract to 2.0 Å, the resolution of this study is being extended to resolve the Fe atoms and to trace the entire polypeptide chain.

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**Note added in proof:** W. V. Sweeney has recently determined by flame emission spectroscopy and colorimetric analysis using 1,10-phenanthroline that *A. vinelandii ferredoxin I* contains six atoms of iron per molecule, based on an extinction coefficient at 400nm of  $27 \text{ mM}^{-1} \text{ cm}^{-1}$ .

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# reviews

## Solar energy boom

D. O. Hall

*Solar Energy: The Awakening Science.* By D. Berham. Pp. 379. (Routledge and Kegan Paul: London, 1979.) £8.95.

WITH worldwide government-sponsored research and development on solar energy in 1979 approaching  $1 \times 10^6$ , it would be very worthwhile for anyone interested in solar energy, however remotely, to read this book. It provides an excellent and well written background to the technologies and personalities of those who have developed and are currently participating in the solar energy boom around the world.

As the United States has the largest and longest running government solar energy budget, about half the world total, its solar energy scene naturally occupies the majority of the book. However, the French solar energy scene is given wide coverage, as they have the third largest programme in the world and have had a sustained effort for many years. Unfortunately the very large Brazilian bio-fuels programme ( $\$300 \times 10^6$  per year) is not dwelt on; nor is the large Saudi Arabian programme ( $\$25 \times 10^6$  per year) nor the other Arab solar energy programmes which are also large. The German solar energy effort is also not discussed, even though their R & D budget is over  $\$30 \times 10^6$  per year; and like the French they have substantial government tax incentives for industries and individuals to install solar energy devices. The United Kingdom solar energy effort (government R & D spending  $\$3 \times 10^6$  per year and no tax incentives) commands a whole chapter. We are credited with having "the thread of originality" (a passively heated school near Liverpool, built in 1961) but "certainly do not believe in trying to solve the problem of solar energy by throwing money at it"—does this sound familiar?

I can thoroughly recommend this book. I read it from cover to cover in a short time but I am probably too interested in solar energy so as to be termed "unusual" or other less kindly epithets. The background work and interviewing which the author (a science writer for UNESCO in Paris) obviously put into the writing of the book comes across clearly. The technical details are kept to a minimum,

and the explanations of the various solar energy systems now operating, or on the drawing board, for houses, factories, swimming pools, satellites, power towers, farms, and so on, are remarkably lucid. It is therefore good for all sections of the community who want to find out what is behind all the jargon which inevitably seems to accompany a technical field. Sometimes one wished for less snippets about the motels and meals and even more about some of the personalities, but the personal flavour of writing is attractive—don't be put off by the first two or three pages (unless of course you like Brittany!) A truly excellent index provides a ready means of finding any topic, person, place, laboratory, company, committee, country (and so on) mentioned in the book. In fact just reading through the index gives one quite an overview of the varied interests and technologies in the field of solar energy.

The final chapter was completed in 1978 and gives an up-to-date view of the status of the implementation of

solar energy programmes in the USA—5,000 solar heated and 50,000 solar hot water houses ( $\$30 \times 10^6$  annual market) with a projection of  $2.5 \times 10^6$  solar heated houses by 1978 ( $\$1 \times 10^9$  annual market). The State of California could not wait for the federal government (which at the end of 1978 offered tax credits up to \$2,200 for each solar energy installation) and became the first State to offer generous incentives—"home owners get a tax credit of 55% up to \$3,000 on a purchase and installation of a solar energy system. Businesses are also given a 25% tax credit if their systems cost more than \$6,000".

The author is not a starry-eyed "solar nut", as he asks searching questions, expresses many doubts throughout the book, and rightly stresses energy conservation techniques right from the start. □

*D. O. Hall is Professor of Biology at King's College, University of London, and chairman of the UK Section of the International Solar Energy Society.*

## Medicinal botany

*Healing Plants: A Modern Herbal.* Edited by W. A. R. Thomson. (Macmillan: London, 1978.) £9.95.

Up to a few years ago very few books on medicinal plants had been written this century for the layman, certainly in English. There was Mrs Grieve's *A Modern Herbal*, published in 1931, as well as some charming if rather less practical books by E. S. Rohde, which came came out about the same time. Mrs Grieve's work covers a wide range of plants, is packed with information, much of which is difficult to find elsewhere, and is a sensible, down-to-earth publication even if it does not contain much information on the chemical constituents of plants (a great deal of work has been done on this in the past fifty years). Nevertheless, I think that when reviewing new books on medicinal plants it is fair to use Mrs Grieve as a standard by which to judge them. The kind of people who will buy and use these new books are probably the same kind as those who bought and

used *A Modern Herbal* in the 1930s.

There seems to be a much wider interest in medicinal plants now than was common even ten years ago. This is no doubt partly a spin-off from the ecology movement but is also the result of the related desire for a simple life, especially among townspeople. Whatever the basis for this interest, it is illustrated by the increase in health food stores selling herbal remedies for a wide range of afflictions. The market for books on medicinal plants is provided with an ever-increasing flow of works of varying merit. The one reviewed here is a typical example and like the curate's egg may be described as good in parts. To start with, it is very attractive to look at; the illustrations—both colour photographs and reproductions of old botanical drawings—are on the whole excellent, as is the printing. But I found the matter contained in the book (and its layout) rather disquieting.

The book is divided into a number of alphabetical lists interspersed with chapters on healing plants and their



active principles, the heritage of folk medicine, and so on. A list is provided of plants under English common-names—not always correct—with the conditions they are held to alleviate; and under latin names with an illustration and general description that would not be very helpful to a non-botanist, and is followed by a list of complaints and illnesses, with the plants used. This list unfortunately does not always tally with the first and is followed by yet another on healing substances and their effectiveness. This last list is about the parts of the plants used, their constituents, and how they are processed. It is the most informative of the sections, but would be more helpful if sources

of both herbal and planting material were given. For example, Asiatic ginseng is listed, but where the authors' expect would-be growers to buy planting material I do not know; so far as I am aware it is only available from Japan. The book would have been far more useful if the information on each plant were all in one place, instead of being scattered throughout. Lists of suppliers—both of the dried herbs and of planting material—would have increased its value enormously. Once again Mrs Grieve wins overall.

**Rosemary Angel**

*Rosemary Angel is Officer in Charge, Museums Division, Royal Botanic Gardens, Kew, UK.*

## Plant breeding for disease resistance

*Plant Breeding for Pest and Disease Resistance.* By G. E. Russell. Pp. 485. (Butterworths: London, 1978.) £25.

DURING the past few years, radical changes have taken place in the strategies used by plant breeders when breeding for resistance to plant pests and diseases. Following the discovery by Biffen in 1907 that resistance to a disease was often conferred by a single gene, known as a major gene, many plant breeders used small numbers of major genes to obtain resistance. It was subsequently found, however, that resistance based on a single major gene, or a few such genes, frequently broke down within a few years of its introduction into a crop plant, due to the pathogen producing new races capable of overcoming that gene. G. E. Russell has been actively concerned in devising new breeding strategies designed to provide durable resistance, and this book reflects his experience and methods of approach.

The first part of the book is devoted to a brief but useful summary of methods used for the control of plant diseases, and to a consideration of the general principles and methods of breeding for resistance. The technical terms and concepts used in plant breeding are clearly defined: though some might not agree with all the definitions offered, they are clear and unambiguous. There is a valuable section on methods, many developed by the author and his colleagues, for the introduction of resistance into commercial crops, and for its assessment when introduced.

Parts II to V are devoted to breeding for resistance to specific groups of pathogenic agents, namely the fungi,

the bacteria, mycoplasmas and viruses, animal pests and parasitic weeds. In each part, a general introduction is followed by a detailed treatment of a few selected cases in each of which substantial progress has been made in the production of resistant cultivars of crop plants. These case histories fulfill two main functions. For the specialist student, they provide a good and usually up to date account of the present position, with adequate references to the literature. For the more general reader, they illustrate very clearly the ways in which the general principles advocated by the author can be applied to obtain durable resistance to almost any type of pest or disease, with modifications in detail rather than in principle to fit any specific case.

In the final part of the book, some general conclusions on the present position and future prospects in the field are set out. The author is hopeful that by the use of major genes, where they are proved to be durable, and by the use of more sophisticated approaches where they are not, it will be possible to control many major pests and pathogens. The casual reader may at first get the impression that it is suggested that breeding is the only satisfactory solution to pest and disease problems, but in the final section, the author is careful to emphasise that in many situations an integrated control programme involving both resistant cultivars, pesticides and biological and cultural control methods may be required.

This book is a very useful addition to the literature of plant breeding and plant pathology. It will be of value both to the student entering this field, and to the more general reader who is seeking an insight into the principles and methods of modern disease and pest resistance breeding.

**J. G. Manners**

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## Applying digital filters

*Digital Filters and their Applications.* By V. Cappellini, A. G. Constantinides and P. Emiliani. Pp. 393. (Academic: London, New York and San Francisco, 1978.) £20.50; \$42.

THE appearance of this volume in a series on *Techniques in Physics* is something of a landmark, for it is only recently that filters have ceased to be studied almost exclusively by electrical engineers, and written about for readers with formal training in that field. The intensive study of two-dimensional filters is also a recent development, spurred on by the need to clean up satellite pictures and by a variety of biomedical and geological needs. In connection with these and related applications, many scientists with no engineering background have been using—and rediscovering—filters to improve their "signals", and the present authors have therefore attempted to compose a text that would appeal to such an audience as well as to engineers. This is no easy task, for the types of presentation likely to satisfy these two categories of reader are rather different, particularly in the handling of mathematical details and in the need to put the material under discussion in context. The solution adopted by the authors, who present a great deal of material with very little comment or explanatory text to help the reader to assimilate it, is not at all satisfactory. It is certainly possible to look up any number of facts about filters, both FIR and IIR, their software and hardware implementations and noise, but it is extremely difficult to get any feel for their power or suitability. The section entitled "Description of some windows" is an example of this: two formulae tell us how to distinguish a Hamming window from a Hanning window but not why two nearly identical windows should coexist. The formulae for numerous other windows are also given, sometimes with some indications about their origin but not always.

This is a disappointing book, therefore, except as an *aide-memoire* for finding formulae and definitions. The authors' object was certainly laudable but for the time being the pair of books on digital signal processing by Rabiner and Gold and by Oppenheim and Shafer (Prentice-Hall, 1975) remain the most useful source of information on these matters.

**P. W. Hawkes**

*P. W. Hawkes is Maître de Recherches at Laboratoire d'Optique Electronique du CNRS, Toulouse, France.*

## Vibrational and electronic spectroscopy

*Symmetry and Spectroscopy: An Introduction to Vibrational and Electronic Spectroscopy.* By M. C. Harris and M. D. Bertolucci. Pp. 550. (Oxford University Press: New York, Oxford and London; 1978.) £15.

A NEW textbook dealing with vibrational and electronic spectroscopy, at third-year undergraduate level, with group theory as the unifying thread, has been needed for some time. This ambitious book goes somewhat further into spectroscopy than I would expect third-year undergraduates to go, from foundations which are not quite deep enough.

The book is ambitious because it aims to be self-contained, assuming little background knowledge (except some quantum mechanics). This has meant difficult decisions as to which theoretical results to explain, which to assume and which to ignore. The first chapter is an extremely readable account of groups and representations which deliberately ignores the theorems

of representation theory (the four rules) and simply states the key result, the formula for decomposing a representation, by *fiat*. The relationship between group theory and the Hamiltonian is not discussed: the student who wishes to know why molecular eigenstates necessarily have symmetry properties must look elsewhere, or simply accept that normal coordinates and molecular orbitals transform as irreducible representations. Without making the book longer, much of this theory could have been included at the expense of chapter two, a sketch of quantum mechanics which adds little to the book and incidentally contains an incorrect interpretation of the superposition principle.

The theory of molecular vibrations and of molecular orbitals provided here is rather elementary compared with the level of the spectroscopy which follows. A verbal definition of a normal coordinate without the equations of motion does not indicate clearly what a normal coordinate is, nor why it can sometimes be determined by symmetry. Similarly, without some idea of an effective one-electron potential, one cannot properly explain the Koopmans theorem nor insist that the molecular orbitals have symmetry properties.

Hückel theory is presented here without any suggestion that the Hamiltonian is not the total molecular Hamiltonian.

Most of the above criticism assumes the educational importance of principles in themselves. Of equal if not more importance for the specialist is the application of principles in puzzle-solving; and it is here that the authors have succeeded brilliantly. Interwoven with the text are many examples of spectra (very well illustrated) from the literature and over 200 problems. The student who completes even a modest fraction of these problems should be able to construct symmetry coordinates and orbitals, determine the states which arise from electronic configurations, and decide which transitions are allowed. In short, he should be able to read the literature of vibronic spectroscopy. The theoretical aspects of this book could easily be supplemented by a lecture course. The strengths of the book lie precisely where formal lectures are often weak—in examples—and for this reason both teachers and students will find it valuable.

M. P. Melrose

M. P. Melrose is Lecturer in Theoretical Chemistry at King's College, University of London, UK.

## Fundamental research in gerontology

*The Biology of Ageing.* Edited by J. A. Behnke, C. E. Finch and G. B. Mowbray. Pp. 388. (Plenum: New York and London, 1978.) £11.93.

AGEING is the next growth industry, perhaps hardly surprising in Western Societies, as two of the three major scourges—famine and pestilence—have been controlled, and the third—war—is unacceptable, at least in theory. About 1 in 6 of our population is over 65. In the United States there are now 22 million people over 65 and early in the next century this figure is expected to increase to 50 millions. Little wonder that the young who have to support the more elderly amongst us are becoming concerned. What do they do with this rapidly increasing army of non-productive geriatrics—parasites on a society which they have helped to build?

Unfortunately, this book does not consider the social problems of ageing but it does deal with most other aspects. It is intended for a general audience but a good knowledge of modern biological theory is an essential prerequisite.

The contributors review most areas of interest in fundamental research in gerontology, particularly ageing in cells and molecules, and ageing in plants and lower animals, but there are also substantial sections on ageing in man and on the effects of hormones on ageing. The book concludes with a section on perspectives. As with any book with 25 contributors, the individual articles vary in quality. Some can be read with pleasure, others are rather heavy going, but all provide good background information and each gives a useful reference list for further reading.

Although ageing and death are the inevitable end-results for most metazoa, we still know very little about the mechanisms involved. It is perhaps the most intellectually challenging problem in biology, yet research on ageing forms only a very small part of the major research programmes. Few universities or medical schools consider ageing to be a basic biological process of sufficient importance to form the basis for organised courses.

This book provides an excellent basis for an introduction to modern research on ageing, but it may be somewhat too detailed to serve as a course book.

L. M. Franks

L. M. Franks is Head of the Department of Cellular Pathology at the Imperial Cancer Research Fund Laboratories, London, UK.

## John Maddox

Croom Helm wish to apologise to Mr John Maddox for the reference to him in connection with recombinant genetics in their book *Directing Technology*. The publishers (Croom Helm) and the Editors (Ron Johnston and Philip Gummert) and the author of the chapter concerned (Dr Edward Yoxen of the Department of Liberal Studies in Science at the University of Manchester) unreservedly withdraw any suggestions of impropriety in Mr Maddox's role in public discussion of this issue.

## High performance liquid chromatography

*Applications of High Performance Liquid Chromatography.* By A. Pryde and M. T. Gilbert. Pp. 255. (Chapman and Hall: London, 1979.) £10.50.

THE authors of all compilations of the applications of any analytical chemical technique have to contend with two basic problems. In the first place only a very small part of the text will be of direct interest to any particular reader, and secondly in the case of any rapidly developing method such as high performance liquid chromatography (HPLC), there is a danger that much of the described methodology will be outdated even during the time required for publication.

The present authors have skilfully avoided both these pitfalls, the first by confining their main coverage to three fields of wide general interest—pharmaceutical analysis, biochemical analysis and methods for monitoring environmental pollution. Some other miscellaneous topics are also treated briefly. The second problem—of current relevance—has been met by providing a very complete review of the literature up to Spring, 1977. Publication delays being what they are at present, this must be considered very good.

A very short but good summary of the relevant theory, a similarly brief account of the currently available instrumentation, and a more extended and valuable section on the practice and principal modes of operation of HPLC lead into the major sections of the book.

Within the selected topics the coverage provided is excellent. For example, the 48 pages devoted to pharmaceutical analysis includes most of the classes of drugs now in general use, from antibiotics to diuretics (including a section on analysis for drugs of abuse). In many cases examples of methods of HPLC analysis for the drug metabolites are included.

The major 61-page section on applications of HPLC to biochemical analysis is particularly thorough, and includes sub-sections on lipids (including steroids and prostaglandins), polycarboxylic acids of metabolic importance, carbohydrates, biogenic amines, amino acids and proteins, nucleic acid derivatives porphyrins, and the common vitamins. It is very encouraging to find some space devoted to HPLC of proteins, as in my view the HPLC of biological macromolecules has lagged behind the much easier HPLC of small

molecules, many of which can readily be fractionated by easier (and much cheaper) methods. The intrinsically high resolution of HPLC methods should be particularly valuable for the separation of large nucleic acid and protein fragments (or even subunits) which are required for the sequencing of the intact molecules.

The brief sections on applications of HPLC to environmental monitoring and various miscellaneous separations, including stereoisomers, provide adequate coverage for their purpose.

The book concludes with a series of appendices listing in considerable detail various types of packings for HPLC columns, and finally with a very use-

ful specific index of compounds mentioned in the text, which greatly enhances its value as a reference book. The scope of this very useful book is illustrated by the 876 quoted references, mostly subsequent to 1970. The production of the book is excellent at its price, and there are very few misprints, although I found the use of the word "colourimetric" throughout the book mildly irritating.

C. J. O. R. Morris

C. J. O. R. Morris is Emeritus Professor of Experimental Biochemistry, formerly at the London Hospital Medical College and Queen Mary College, University of London, UK.

## Molecular photoelectron spectroscopy

*Photoelectron Spectroscopy and Molecular Orbital Theory.* By R. E. Ballard. Pp.192. (Adam Hilger: Bristol, 1978.) £18.

THE author of this book has appreciated the value of molecular photoelectron spectroscopy as a teaching aid in chemistry. Other books on the subject have tended to emphasise its contribution to the advancement of knowledge and have been directed primarily at researchers. He recalls the late Professor C. A. Coulson's remark about the impact of photoelectron spectroscopy: "The single result of most significance is the complete vindication of the molecular orbital description of a molecule". Molecular orbital theory is taught throughout university chemistry schools, sometimes with the clarity which the late Professor Coulson used to demonstrate, all too often, however, as a beautiful mathematical theory; that in some ways is good for the undergraduates to master, but which many find sterile because of an over-emphasis on exactitude. The aim of the present book clearly is to demonstrate that photoelectron spectroscopy and especially helium I photoelectron spectra of vapours have an important part to play in introducing undergraduates rather painlessly to some of the essential concepts of molecular orbital theory.

In order presumably not to overburden the student, a number of topics which a specialist would think essential have been omitted; notably no discussion is given of the interpretation of spectra based upon ionic states, to which reference must always ultimately be made. The author is content to emphasise only the relationship between occupied orbitals and the

bands in the photoelectron spectra. This is attempted by assembling the spectra for a rather limited range of simple molecules falling into groups of increasing complexity and then discussing these in some detail. These comparative chapters are preceded by a more general introduction to the necessary molecular orbital concepts. Some use has been made in the comparative chapters of the computer-generated drawings of Jorgensen and Salem (*The Organic Chemists' Book of Orbitals*, Academic: New York, 1973) to provide an insight into the geometrical factors which are often implicit in the details of the photoelectron spectra. This is often a most revealing juxtaposition and the reviewer feels that even more use could have been made of this to advantage. The detailed comparisons are quite restricted in their coverage, ranging from diatomics, through triatomics, molecules related to ethylene, and some tetrahedral and octahedral molecules. Within these groupings are, however, to be found a number of examples of substances whose electronic structure will have been a puzzle at one time or another to most chemistry undergraduates.

The importance of the finer detail in the spectra is not treated in depth. The section on vibrational fine structure is perhaps less well illustrated in certain respects than other areas, both with regard to selection of examples and more particularly the quality of reproduction of some of the spectra. In this area in particular spectral resolution and the fineness of detail which can be read into many spectra are of fundamental importance; their proper discussion requires both well reproduced examples and a proper treatment of the concept of the ionic state manifold, which, as remarked above, is a significant omission.

D. W. Turner

D. W. Turner is Reader in the Physical Chemistry Laboratory, University of Oxford, UK.



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Editors: J. SKODA AND P. LANGEN, Prague, Czechoslovakia

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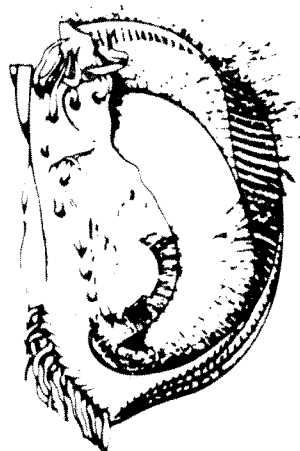
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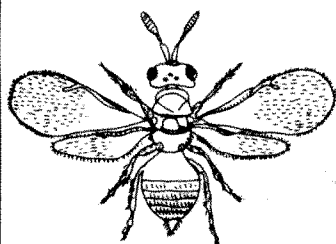
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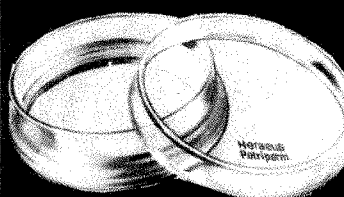
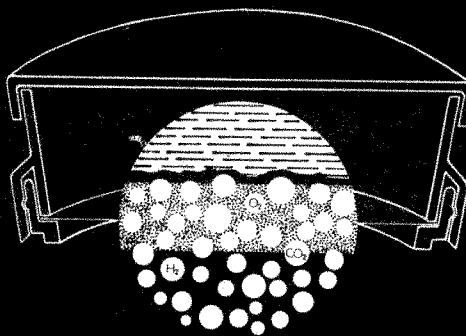


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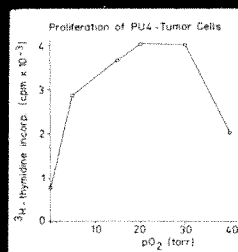


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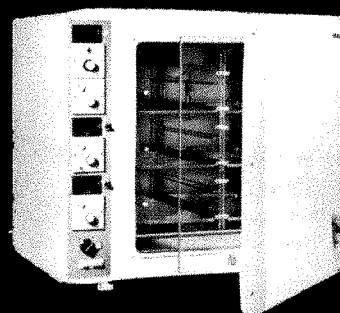
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# newly on the market

**Optical scanner.** The new Stahl Research optical scanner can scan biological microsamples with high accuracy. A resolution of  $0.2\text{ }\mu\text{m}$  per pixel over a  $50\text{-}\mu\text{m}$  field is attained, resulting in a grid of  $256 \times 256$  pixel images. The Model 502 image analysis optical scanning microscope system guarantees  $0.2\text{ }\mu\text{m}$  overlay reproducibility with each subsequent scan. The microprocessor controlled system utilises oscillating mirrors and PMT detection to achieve a  $s/n$  ratio better than 46 dB. Included is a high precision X-Y stage and provision is made for a host of image processing algorithms. **Circle No. 84 on Reader Enquiry Card.**



The Stahl Research optical scanner

**Multi-wavelength photometer for HPLC.** The Du Pont 860 absorbance detector is a compact UV/visible photometer designed for high sensitivity detection of chromatographically separated components that absorb light at the mercury emission lines in the 254 to 546 nm wavelength region. The device can detect sample components at picogram levels. Model 860 uses optical filters and a stable, low pressure mercury lamp source with a phosphor-coated tip. A two-position mounting permits easy conversion from 254 to 284 nm. The flow-cell design permits sensitivities to 0.002 absorbance units full scale. Sample absorbance is continuously displayed on a digital meter for visual monitoring and recording. **Circle No. 85 on Reader Enquiry Card.**

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**Infra-red detectors.** Plessey announces a new range of fast-response uncooled infra-red detectors designed for general purpose infra-red laser studies, where the detection of low power fast infra-red pulse chains is required. These new devices, designated the PLT 411F series, have as detector element a single lithium tantalate crystal which has high voltage but for maximum mean power handling capability a pyroelectric ceramic element can be offered.

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**Liquid scintillation system.** The Packard Tri-Carb 460C, is an advanced microprocessor-based liquid scintillation system which dramatically increases the quality of data that can be obtained in cancer research, disease prevention, genetics, microbiology and other life science investigations. It features an integrated video display showing test procedures and results, a system controller and a built-in printer to record information on a report form. The system can handle as many as 460 samples. It has cassettes, or rack-like carriers which hold up to 10 samples each.

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**Cellulose acetate electrophoresis system.** A purpose built cellulose acetate system that produces consistently good results for a wide range of clinical applications has been produced by Shandon. Applications include the separation, identification and quantitation of serum proteins, haemoglobins, haptoglobins and glycoproteins. The system incorporates a newly developed cellulose acetate membrane, Celagram II, which provides sharper separations and shorter running times than previous membranes and eliminates the need for a final sticky clearing bath.

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**Luteinising hormone assay.** The new radioimmunoassay kit for Luteinising Hormone (LH) in human serum, from Amersham Corporation covers the range  $0\text{--}300\text{ mIU ml}^{-1}$ . Good performance throughout the shelf-life (normally 4–7 weeks from date of shipment) of the kit is ensured by the use of ion-exchange paper to partially purify the tracer prior to each assay. The Amersham LH RIA kit has been clinically tested.

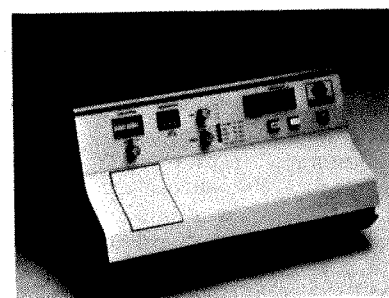
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**Luminometer.** LKB-Wallac is introducing a new instrument for luminescence measurements. The Luminometer 1250 is the basic instrument comprising the measuring chamber with detector and the electronics unit. The detector is a photomultiplier which gives the instrument high sensitivity. The spectral response covers both chemiluminescence and bioluminescence. Various output devices can be used with the 1250. LKB-Wallac can supply a digital display and printer unit or a potentiometric recorder.

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**Nanometre particle sizer.** The Coulter Nano-Sizer determines average particle size in suspensions and emulsions in the overall range  $0.04\text{--}3.0\text{ }\mu\text{m}$ . This, together with an index of the width of the size distribution, is presented typically within 2–4 min with no operator attention, calculation or calibration. The built-in micro-computer ensures that no calculations are required and alleviates the necessity for calibration. Results are absolute and are displayed digitally to three significant figures. Areas of application include paints, pigments, latices, pharmaceuticals, emulsions of all types, carbon blacks and other colloidal materials.

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The Coulter Nano-Sizer

**Ultrasonic pipette and glassware cleaner.** The Branson Sonifier ultrasonic pipette and glassware cleaner is a new accessory to the Sonifier cell disruptor product line. The cleaner, comprised of an ultrasonic generator and a stainless steel cleaning tank, provides fast, efficient cleaning of all sizes of pipettes, glassware, and other laboratory instruments. No pre-soaking is necessary and several cleaning solutions are available.

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**Audible radiation monitor.** The Radia-tron 'Bristol Bleeper', developed in conjunction with the Physics Department Bristol General Hospital, is a convenient and simple method for safeguarding personnel against ionising radiation. The 'Bristol Bleeper' is only slightly larger than a pen and slips easily into a pocket, where it is held firmly by the strong stainless steel clip. The monitor operates over a wide range of radiation energies. It operates continuously giving a bleep every 10 min for normal background radiation increasing, with dose rate, to a continuous tone when high radiation doses are present. Typical endurance is 1 year using readily available batteries.

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The Bristol bleeper

**Light pollution rejection filter.** Celestron now offers a series of LPR filters that make bright city, light-polluted skies, appear dark by rejecting radiation from mercury and sodium type lights. This will be of great value to the amateur astronomer who will be able to make deep sky photographs from sites in built-up areas.

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**Computing integrator.** The Spectra Physics SP4100 computing integrator is microprocessor controlled, has a full Alpha Numeric keyboard and LED display and an integral Printer Plotter. BASIC is standard in the SP4100 with extensive built-in programming which can be easily reprogrammed by the user. The most often used data reduction methods in chromatography are all preprogrammed in ROM (Read Only Memory) and can be implemented with just a few key strokes. Built-in BASIC programming capability allows the user complete flexibility to modify an existing data reduction method, format his own report or develop other custom applications. The printer plotter prints at 24 characters per second in black on white, and can 'chart reverse' to do X-Y plotting.

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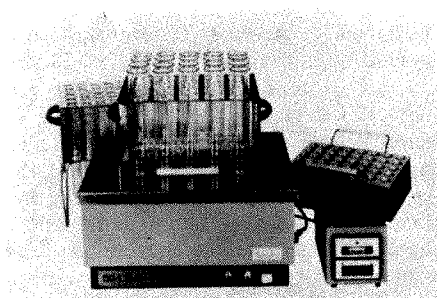
#### Monoclonal materials for B cell studies.

Sera-Lab produce three monoclonal antibodies directed against sheep red cells. The antibodies have been produced by the hybrid myeloma technique of Kohler and Milstein and are serologically distinct belonging to the mouse immunoglobulin classes IgG<sub>1</sub>, IgG<sub>2</sub> and IgM. For workers involved in the separation of human B lymphocytes for tissue typing and histocompatibility tests prior to transplantation, two different sera are available directed against the HLA-DR markers. The antibody MAS 020 is a non-complement fixing antibody and can be used for the preparation of enriched B cell suspensions for subsequent typing using complement dependent sera. Serum MAS 019 is a complement-dependent serum directed against B cells, which can be used for separation methods and as a good positive control in cytotoxicity tests and similar reactions.

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**Block digester for wet digestion/oxidation techniques.** Techne Inc. have developed a block digester system designed for sample preparation prior to analysis of grain, food products, soil, water pollutants, chemicals etc. The block digester is an alloy block heater system designed to heat samples at temperatures up to 450 °C for wet oxidation or acid digestion of material for assay, particularly of protein by the Kjeldahl method.

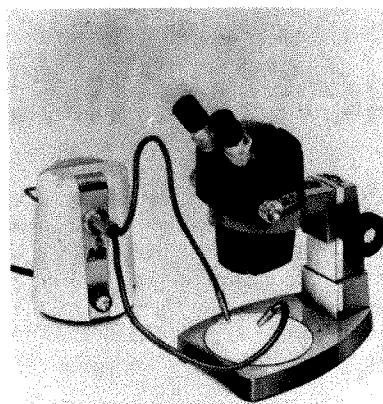
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Techne block digester

**HPLC chromatographic packings.** Du Pont present Zorbax NH<sub>2</sub> columns and Zorbax bulk liquid chromatographic packings. Zorbax NH<sub>2</sub> is a multipurpose column that can be used for high performance separations under normal, reversed phase, and ion exchange chromatographic conditions. This polar bonded column expands the range of Zorbax applications to include separations of materials such as carbohydrates, organic acids, and other polar organic compounds, including preservatives, antioxidants and pesticides. Zorbax bulk liquid chromatographic packings are spherical with a diameter of 7-8 µm.

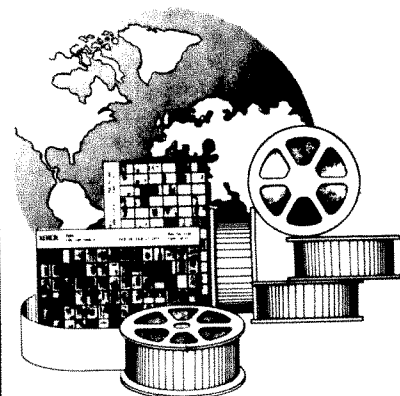
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**Fibre optic illuminator for stereo microscopes.** Reichert-Jung UK in conjunction with American Optical Corporation announce the model 11-80 Fibre Optic Illuminator suitable for use within stereo microscopes from any manufacturer. The 11-80 incorporates a quartz halogen lamp (colour temperature 3,400 K) and its output is directed at the specimen through self-supporting fibre optic light guides. Single and branched guides are available, permitting accurate pinpoint illumination of the specimen. The unit incorporates a fan for cooling and has a neat cable storage.

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650(A)

### UMIST

#### UMIST Pollution Group

The members of the UMIST Pollution Group are responsible to the Assistant Director of the jointly organised UMIST/University of Manchester Pollution Research Unit, which consists of a multi-disciplinary group of staff whose interests cover the Technical, Legal, Economic and Social aspects of pollution. The one-year M.Sc. Course in Pollution and Environmental Control has been accepted by the S.R.C. as suitable for the tenure of its Advanced Course Studentships. Applications are invited for the following posts based at UMIST.

#### Lecturer (Reference UPG/68/AD)

The Scientific, Technical and Engineering activities of the UMIST Group are undergoing expansion, and a lecturer is now required. The successful applicant will be expected to make significant contributions to the M.Sc. Course as well as to undergraduate courses in other departments, and to pursue his/her own research interests, both within the Group as well as in collaboration with other departments. Applicants will be considered primarily on the basis of their personal achievements rather than on their conformity to a prescription of suitable interests, but those offering expertise in the fields of Public Health Engineering or Noise Pollution and its Control may well offer the most effective means of complementing the existing staff interests.

Salary will be on the scale £3,883 to £7,754 per annum (subject to revision from October 1978).

#### Project Officer (Reference UPG/69/AD)

In addition to the normal academic pursuits the unit operates a service to industry in topics falling within the pollution and environmental control field and a vacancy now exists for a full time Project Officer to be responsible to the Assistant Director for the day-to-day operation of this service. Versatility and competence in the practical skills necessary to the performance of this service as well as enthusiasm in consolidating opportunities to create new work will be important qualities in the successful candidate. Some involvement in longer term projects will be encouraged in order to enable the Project Officer to maintain his acquaintance with technical developments.

Salary will be on the scale £3,883 to £6,555 or £6,317 to £7,754 per annum subject to qualifications and experience (subject to revision from October 1978).

Application Form and Conditions of Appointment may be obtained from the Registrar, UMIST, P.O. Box 88, Manchester M60 1QD. Appropriate reference should be quoted. Informal enquiries should be addressed to Dr R. F. Griffiths. 640(A)

### UNIVERSITY OF LONDON INSTITUTE OF OBSTETRICS AND GYNAECOLOGY Hammersmith Hospital

A vacancy exists in the Research Laboratories of the Institute of Obstetrics and Gynaecology, Hammersmith Hospital, for a graduate biochemist, preferably with previous experience in prostaglandin work or tissue culture. This is a three-year project to investigate the role of prostacyclin in fetal growth retardation and will lead to an M.Phil./Ph.D. degree. There will be some collaboration with the Department of Clinical Pharmacology, Royal Postgraduate Medical School. The starting date would be June/July 1979. Please send one copy of curriculum vitae to Professor M. G. Elder, Institute of Obstetrics and Gynaecology, Hammersmith Hospital, Du Cane Road, London W12 0HS by Friday May 18. 645(A)

### THE MIDDLESEX HOSPITAL MEDICAL SCHOOL (University of London) POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited for a three-year appointment supported by the Science Research Council, to work on adhesion and locomotion of tissue cells. Candidates should possess a Ph.D. degree, or expect to obtain one this year. Salary £4,857 per annum, rising to £5,340 per annum (inc. London Allowance). Applications, including curriculum vitae and the names of two referees to Dr David Gingell, Department of Biology as Applied to Medicine, The Middlesex Hospital Medical School, London W1P 6DB. 646(A)

### THE UNIVERSITY OF HULL DEPARTMENT OF BIOCHEMISTRY BIOLOGICAL CHEMIST/ BIOCHEMIST

Applications are invited for a **Post-doctoral Research Assistant** for an attractive and interesting research project concerning the structure and function of iron-binding compounds of the mycobacteria. The position which is created by a grant from the SRC also provides for technical assistance. Candidates should have or expect to have obtained their Ph.D. before the starting date.

Salary will be on the lower part of the scale £3,883 to £4,882 (under review).

### BIOCHEMIST/ MICROBIOLOGIST

Applications are also invited for a graduate **Research Assistantship** for a related project involving mycobacteria of medical and clinical importance. The position is created by a grant from the MRC and will involve some co-operation with the National Institute for Medical Research. Candidates should have or expect to obtain a good honours degree in an appropriate subject.

Salary will be on the scale £3,384 to £3,883 (under review).

Both positions are to start October 1, 1979 and are for three years (subject to satisfactory progress); they will be under the direction of Dr Colin Ratledge.

Applications should give full details of education, qualifications and research experience, where appropriate, together with the names of two referees and should be sent by May 28, 1979 to Dr C. Ratledge, Department of Biochemistry, The University of Hull, Hull, HU6 7RX from whom further details of both positions may be obtained. 601(A)

## Centre for Applied Microbiology & Research, Porton Down, Therapeutic Products Laboratory

The Therapeutic Products Laboratory at the new Centre is concerned with the research, development and production of microbial enzymes and human proteins for clinical use. The Laboratory already manufactures Asparaginase that is widely used for the treatment of leukaemia and has other enzymes in various stages of development. The manufacture of human growth hormone will begin shortly and a capability is being developed for the large-scale freeze-drying of therapeutic products in vials. The Posts described below are likely to suit the person who wants to play a well-defined and worthwhile role in a team that has the common purpose of producing new therapeutic products for clinical use.

### Microbial Screening Section

## Senior Grade Microbiologist

The scientist appointed will be head of this section and responsible for:

- (i) Research, development and application of screening and selective procedures to find new (natural or genetically contrived) microbial sources for therapeutic enzymes.
- (ii) Production of cell paste from promising strains for enzyme purification, characterisation and biological evaluation.
- (iii) Supervision of quality control tests for microbial contamination.

**Qualifications:** Honours degree in Microbiology or Biochemistry and a wide experience in microbiological research. Experience in fermentation technology would be an advantage.

### Process Development Section

## Biochemist or Chemist

The successful candidate will be appointed as a Basic Grade Microbiologist and will be responsible for:

- (i) Development of processes for the purification of microbial enzymes and other proteins to the requirements of the Medicines Act.
- (ii) Research into many problems related to the manufacture of pharmaceutical products.
- (iii) Characterisation of enzymes with respect to substrate specificity and stability after storage and freeze-drying.

**Qualifications:** Honours degree in Biochemistry/Chemistry and preferably at least three years experience in protein fractionation and enzymology.

### Protein Chemistry Section

## Biochemist or Pharmacologist

The successful candidate will be appointed as a Basic Grade Microbiologist and will be responsible for the following research programme.

- (i) Investigation into the effects of chemical modification on the biochemical and biological properties of proteins with a view to improving the efficacy of therapeutic products in man.
- (ii) Design and operation of a wide variety of animal tests for the evaluation of therapeutic products.

**Qualifications:** Honours degree in Biochemistry/Pharmacology and preferably at least three years postgraduate research experience.

### Therapeutic Enzymes Section

## Biochemist or Chemist

The successful candidate will be appointed as a Senior Medical Laboratory Scientific Officer and will be responsible for:

- (i) Large-scale purification of Asparaginase and other microbial products from cell extracts.
- (ii) Development and performance of quality control tests to comply with the requirement of the Medicines Act.

**Qualifications:** University degree or HNC in Biochemistry or Applied Biology or Chemistry and experience in protein chemistry and separation techniques.

If any candidate should require further details of the above posts they should contact Dr J. E. Benbough, Deputy Director, Therapeutic Products Laboratory (Idmiston 610391) and he will be pleased to arrange visits to C.A.M.R. by candidates.

Applications to Mr Paul Murphy, Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire SP4 0JG.

N.H.S. terms and conditions of service will apply to all these posts.

### Analytical Chemistry Section

## Two posts for Chemists or Biochemists

The Senior post will be filled as a Senior Medical Laboratory Scientific Officer. The successful candidate will be responsible for:

- (i) Organising and conducting analysis of amino acids, trace metals, enzymes and a wide variety of other substances.
- (ii) Compiling documents relating to the quality control of materials used for the manufacture of therapeutic products.
- (iii) Developing rapid methods for assay of enzymes and hormones.

**Qualifications:** University degree in Chemistry or Biochemistry or F.I.M.L.S. and several years experience in analytical work.

The second post in this section will be filled as a Medical Laboratory Scientific Officer. The successful candidate will be responsible for the operation of a wide variety of analyses in biological samples.

**Qualifications:** HNC (or equivalent) and preferably some experience in biochemical or clinical analysis.

### Central Services Section

## Senior Medical Laboratory Scientific Officer

The successful candidate will be responsible for:

- (i) Organisation of services for the Therapeutic Products Laboratory.
- (ii) Preparation of sterile areas for aseptic operations.
- (iii) Organisation and operation of sterile-filtration of bulk therapeutic products.
- (iv) Organisation of quality control tests on finished clinical products and the packaging of vials for distribution.
- (v) Management and training of Ancillary workers.

**Qualifications:** F.I.M.L.T. or the equivalent and experience in medical (or pharmaceutical) laboratory practices. We are looking for someone who is sufficiently versatile and well-organised to execute a wide range of operations used in pharmaceutical manufacture.

### Human Proteins Section

## Medical Laboratory Scientific Officer

The successful candidate will provide technical assistance for:

- (i) Purification of Human Growth Hormone for clinical use.
- (ii) Research and development work on other human proteins.
- (iii) Quality control tests involving protein separation techniques and immuno-assays.

**Qualifications:** HNC (or equivalent) in Applied Biology or Biochemistry or Chemistry.

### Freeze-Drying Section

## Medical Laboratory Scientific Officer

The successful candidate will provide technical assistance for:

- (i) Small- and large-scale freeze-drying of pharmaceutical products such as enzymes and vaccines.
- (ii) Operation and maintenance of freeze-dryers and related scientific instruments.
- (iii) Experimental freeze-drying of new products involving biophysical characterisation.

**Qualifications:** University degree or HNC (or equivalent) in Chemistry, Physics or Applied Biology. Some mechanical aptitude would be an advantage.

### Salary Scales:

Senior Grade Microbiologist .....	£5,451	to	£6,837
Basic Grade Microbiologist (with honours degree 1st or 2nd class) .....	£3,486	to	£4,899
Senior Medical Laboratory Scientific Officer .....	£4,347	to	£5,769
Medical Laboratory Scientific Officer .....	£3,261	to	£4,680

Closing date for applications: May 25, 1979

PH  
LS

Public Health Laboratory  
Service Board.

676(A)



## FACULTY OPENINGS exist at the Higher Institute of Technology (Brack) Socialist Peoples Libyan Arab Jamahiriya

in:

### ENVIRONMENTAL TECHNOLOGY DEPARTMENT

**Applied Statistician/Biometrician:** M.Sc., Ph.D., to teach basic statistics applied to biology, ecology and medicine.

**Analytical Chemist:** M.S., Ph.D., Teaching and practical experience in general analytical and instrumental methods, or experience in water or environmental analysis.

**Environmentalist:** M.S., Ph.D., In environmental studies and geographical aspects. Teaching and practical experience required.

**Ecologist:** M.S., Ph.D., Experience in warm climates preferred. Interest in field work is essential.

**Environmental Engineer:** M.Sc., Ph.D., or Engineering Degree. To teach environmental engineering relevant to a developing country. Teaching and practical experience in "warm" climates is an advantage.

### Technologists, Technicians/Demonstrators:

1. With degree or extensive experience in preparing and teaching ecology laboratory practicals. Knowledge of electronics is helpful.
2. Experience in analytical chemistry and instrumental methods of analysis. Environmental Microbiologist with Water, Soil and Air analysis experience.

### Medical Technology Department

**Hematologist - Serologist:** M.S., Ph.D. or Certified Medical Technologist. To teach Hematology and Serology. Teaching and practical experience desired.

**Demonstrators, Technologists, Technicians:** For teaching laboratory in Biochemistry, Clinical Chemistry, Hematology and Medical Microbiology.

### Food Technology Department Lecturers

**Food Analytical Chemist:** M.Sc., or Ph.D. with teaching and practical works experience.

**Food Nutritionist:** B.Sc., or M.Sc., with teaching and laboratory experience.

**Technologist/Demonstrator and 2 Technicians:** B.Sc./HND or equivalent qualifications for laboratory demonstration.

All applicants must be fluent in spoken English, the language of instruction. Appointments depend on qualifications of appointees. No age limit exists on applicants. Fringe benefits include free air conditioned housing, medical services, transportation and terminal gratuity.

Applications should be addressed to:

**The Dean  
Higher Institute of Technology  
Lyster Campus, Hal-Far, Malta.** W94(A)

Centre for Applied Microbiology & Research  
Porton Down  
Special Pathogens Reference Library

## Top Grade Microbiologist

Applications are invited from Scientists/Microbiologists with an appropriate higher qualification for this post. The laboratory is concerned with the diagnosis of severe human microbial infections, particularly those caused by viruses such as Lassa, Marburg and Ebola. It is also actively concerned with research on such viruses and the diseases they cause. Experience in the handling of dangerous pathogens and of work in high containment laboratories will be an advantage.

Salary on NHS Top Grade Biochemist/Physicist scale (£8,877 to £10,347). Other terms and conditions of service generally as for appointments in the NHS.

The laboratory may be visited by arrangement with the Director, Dr D. I. H. Simpson. Telephone 0980-610391.

Full details of this post may be obtained from Mrs M. Bushby, Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wilts SP4 0JG, to whom applications together with curriculum vitae, stating date of birth, qualifications, experience and published work, and naming 3 referees should be sent to arrive not later than May 18, 1979. 602(A)

PH  
LS

Public Health Laboratory  
Service Board.

### AUSTRALIAN NATIONAL UNIVERSITY

John Curtin School of  
Medical Research  
DEPARTMENT OF  
PHARMACOLOGY  
RESEARCH FELLOW

Applications are invited for appointment to the above-mentioned post. The successful applicant will be expected to pursue research under the general direction of Professor D. R. Curtis into synaptic mechanisms within the mammalian central nervous system at a cellular level, and to be familiar with the relevant anatomical, biophysical, and pharmacological techniques. Further information may be obtained from Professor Curtis, in the University.

Appointment will be for two years in the first instance with the possibility of extension to a maximum of five years.

**CLOSING DATE: JUNE 15, 1979.** Salary on appointment will be according to qualifications and experience within the range: \$A15,786 to \$A20,606 per annum. Present exchange rates are \$A1 : 56p.

Reasonable appointment expenses are paid.

Superannuation benefits are available for applicants who are eligible to contribute. Assistance with finding accommodation is provided for an appointee from outside Canberra.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should obtain the further particulars from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. 630(A)

### UNIVERSITIES OF MANCHESTER AND LIVERPOOL

Universities Research Reactor  
Risley, near Warrington

### EXPERIMENTAL OFFICER (Assistant Analyst) IN THE ACTIVATION ANALYSIS SERVICE

Applications are invited for the post of EXPERIMENTAL OFFICER (ASSISTANT ANALYST) in the Activation Analysis Service of the Universities Research Reactor. The successful candidate will be expected to plan and carry out trace element analysis of a wide range of materials submitted to the Service by Industrial and University research workers. The techniques usually involved are those of thermal neutron activation; other radiochemical techniques are used when necessary. Facilities available include the 300kW nuclear reactor on the site, a fully equipped Radiochemical Laboratory, comprehensive detection and nucleonic equipment. Candidates should have a sound knowledge of inorganic chemistry; experience in inorganic analysis or radiochemistry would be an advantage, together with at least pass degree or equivalent in chemistry. Salary range: £3,384 to £5,604 per annum (under review) U.S.S. Particulars and application forms (returnable by May 25) from the Registrar, The University, Manchester M13 9PL Quote ref: 92/79/N.

666(A)

## Administrative Appointments for Scientists

Science graduates interested in a career in research administration—giving opportunities to be involved with the latest developments in medicine and biology—are invited to apply for appointment as a Scientific Administrative Officer in the Council's London office. The Council has an annual budget of some £60m and is the main government agency for promoting medical research.

The successful candidate is likely to be aged between 30–40, with some experience both of biomedical research at a post-doctoral level and of administration, and with broadly informed interest in science and medicine. The job carries key responsibility for day-to-day liaison between the Council and research workers, both in the Council's own research establishments and in Universities, Medical Schools, Hospitals and Research Institutes.

The Council's headquarters staff are appointed on terms and conditions analogous to those of the Civil Service. Subject to satisfactory completion of a 2 year probationary period, the appointment will be permanent. The salary scale (Principle Scientific Officer grade) offered is £6,609 to £8,461 (currently under review) plus £524 London Weighting with superannuation provision; and there is opportunity for promotion.

Further information and application forms may be obtained by writing or telephoning to Miss Margaret Gale, Medical Research Council, 20, Park Crescent, London W1N 4AL (01-636 5422 Ext. 35); the closing date for applications is May 14, 1979.

# MRC

Medical Research Council

680(A)

### UNIVERSITY OF THE WEST INDIES—TRINIDAD

Applications are invited for the following posts:—

**LECTURER/ASSISTANT LECTURER BIOCHEMISTRY**  
DEPARTMENT OF BIOLOGICAL SCIENCES with interest and training in Nutrition, Chemical Microbiology, Plant Biochemistry or Natural Products.

**LECTURER/ASSISTANT LECTURER SOIL PHYSICS**  
DEPARTMENT OF SOIL SCIENCES with experience and interest in applied aspects of soil water relations, tillage, drainage, irrigation and land reclamation.

**RESEARCH FELLOW/JUNIOR RESEARCH FELLOW** (two posts)  
DEPARTMENT OF SOIL SCIENCES with postgraduate qualification in Soil Physics or Agricultural Engineering and interest in management of clay soils for production of annual food crops. Appointee may be stationed in Guyana.

Salary scales (1977/8 under review)  
Lecturer/Research Fellow—TT\$19,071 to TT\$29,799 per annum. Assistant Lecturer/Junior Research Fellow—TT\$15,480 to TT\$16,974 per annum (£1 sterling equals TT\$5.02). F.S.S.U. Unfurnished accommodation if available at 10 per cent or furnished at 12½ per cent or housing allowance of 20 per cent or pensionable salary. Family passages. Study and Travel Grant. Detailed applications (two copies) with curriculum vitae and naming three referees to Secretary, U.W.I., St Augustine, Trinidad, as soon as possible. Applicants resident in the U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address.

607(A)

### UNIVERSITY OF HONG KONG

**LECTURESHIP/ ASSISTANT LECTURESHIP IN BIOCHEMISTRY**

Applications are invited for the post of Lecturer/Assistant Lecturer in Biochemistry. Applicants from any field of research endeavour will be considered; experience in immunochemistry or in teaching dental students will be an advantage, but is not essential.

Annual salaries (superannuable) are: Lecturer HK\$63,540 by 4,260 to 72,060; Assistant Lecturer HK\$46,260 by 4,320 to 59,220. (£1 = HK\$10.70 approx). A medically-qualified appointee who has completed his pre-registration year will be appointed as a Lecturer at the minimum point of the scale. Starting salary will depend on qualifications and experience.

At current rates, salaries tax will not exceed 15% of gross income. Housing at a rental of 7½% of salary, education allowances, long leave and medical benefits are provided.

Further particulars and application forms may be obtained from the Association of Commonwealth Universities (Apps), 36 Gordon Square, London WC1H 0PF, or the Assistant Secretary (Recruitment), University of Hong Kong, Hong Kong.

Closing date for applications is June 30, 1979.

672(A)

## UNIVERSITY OF MAINE

With the establishment of the Center for Marine Studies, the University of Maine is placing priority emphasis on the development of a strong, competitive research and graduate education program in marine resources. Nominations and applications are invited for the following positions.

### CHAIRPERSON DEPARTMENT OF OCEANOGRAPHY

The Oceanography Department, a graduate department offering M.S. and Ph.D. degrees, is primarily housed at the University's marine laboratory at Walpole Maine. It currently has a faculty of 8. Present research activities include ecology of sub-boreal estuarine organisms, environmental biology and geochemistry, aquaculture and geological oceanography.

#### Responsibilities:

to serve as chief administrative officer, and develop a coordinated research program in physio-chemical estuarine processes.

#### Qualifications:

- (1) innovative leadership and administrative capability,
- (2) continuing scholarly achievements,
- (3) evidence of consistent success in funded research,
- (4) earned doctorate.

Academic appointment as Associate or Professor, tenure track. Salary open within University constraints.

### FISHERIES POPULATION DYNAMACIST DEPARTMENT OF ZOOLOGY

The Zoology Department, with a current faculty of 18, is primarily housed on the Orono campus, and includes the Cooperative Fisheries Unit. Ten of the faculty have research interests in marine or freshwater biology.

#### Responsibilities:

to develop a graduate education and research program on population dynamics of fishes.

#### Qualifications:

- (1) earned doctorate,
- (2) additional professional experience in marine fisheries biology desirable.

Academic appointment as Assistant Professor, initially one year with probability of additional 2-4 year University commitment.

### MIGRATORY FISH BIOLOGIST MIGRATORY FISH RESEARCH INSTITUTE

The Migratory Fish Research Institute is an interdisciplinary group of primarily Zoology faculty whose research interests focus on the biology of migratory fishes.

#### Responsibilities:

to collaborate in research on oceanic and estuarine migrations of larval and juvenile eels, and to assist in research proposal development.

#### Qualifications:

- (1) earned doctorate,
- (2) training and experience in fish migration and orientation preferred, and,
- (3) marine background or orientation.

Research appointment, initially one year with probability of second year continuation and possibility of third year depending on extramural program funding.

### MARINE STATION ADMINISTRATIVE OFFICER

The Ira C. Darling Center is the marine laboratory of the University of Maine. It is located on a 152 acre site on the Damariscotta estuary and includes Lowe's Cove. The facility comprises 10,000 square feet of laboratory and teaching space, including an Aquaculture Center, a developing conference center, a library, dockside facilities, and a fleet of small vessels.

#### Responsibilities:

to serve as the Chief Administration officer of a semi-isolated marine facility in support of educational and research programs, to be responsible for budget development and control, and for an expanding promotional/developmental activity.

#### Qualifications:

- (1) extensive administrative experience,
- (2) evidence of ability to communicate effectively with the public, and,
- (3) experience in sea-oriented organisation including logistics, desirable.

Salary \$16,000 to \$18,000.

Applicants should send complete resumes, samples of publications, and names of three referees, by May 15, 1979, to:

Secretary, Search Committees  
Center for Marine Studies  
Room 14, Coburn Hall  
University of Maine at Orono  
Orono, ME 04469 U.S.A.

The University of Maine is an Equal Opportunity/Affirmative Action Employer.

W95(A)

# Opportunities in Pharmacological Research

The Pharmaceutical Division of Reckitt and Colman—a rapidly growing research-based unit requires scientists to consolidate and further expand its already successful biological research teams.

Our analgesic, immuno-inflammatory and CNS research teams have already achieved considerable success and have been associated with several recent therapeutic successes (e.g. buprenorphine, etorphine, diprenorphine, fenclofenac and sodium valproate). To continue and extend these successes we intend to expand our biological research teams in all of these projects. In addition, the increasing demands by drug regulatory authorities around the world has also necessitated the enlargement of the pharmacological development teams concerned with the acute safety and endocrinological effects of prospective drugs.

Such expansion will present several types of opportunities to prospective candidates:- to post-doctoral scientists with specialist experience either in academia or industry and leadership ability and also to newer graduates with a minimum of good honours degree and a sound knowledge of pharmacological principles.

The importance Reckitt and Colman continue to attach to pharmaceutical research will be reflected in the salaries offered with these appointments. Contributory pension and bonus schemes are in operation. For an application form or for further information concerning these appointments please write in complete confidence to:

Miss N. V. McGeown, Personnel Officer,  
Reckitt and Colman Pharmaceutical Division,  
Dansom Lane, Hull HU8 7DS.



## Reckitt & Colman

677(A)

# ETH Zürich

The Swiss Federal Institute of Technology in Zürich has an opening for the

## Chair of Applied Microbiology

The new professor is responsible for teaching and research in biotechnology, especially in its application to biologically mediated processes in natural aquatic systems and continuous fermentations with mixed microbial biocenoses such as in water and waste treatment plants. It is desirable that the applicant be ready to act as a head of the department of biology of the Federal Institute for Water Resources and Water Pollution Control associated with the Swiss Federal Institute of Technology.

The successful applicant should have several years of relevant experience and proven ability to perform and direct research. He will be expected to teach, at both undergraduate and graduate levels, and to cooperate with various research groups.

Applications, with curriculum vitae and list of publications, should be sent by May 31, 1979, to Prof. H. Ursprung, President, Swiss Federal Institute of Technology, CH-8092 Zurich.

W87(A)

UNIVERSITY OF FRIBOURG  
Switzerland

Applications are invited for the post of

### PROFESSOR OF ORGANIC CHEMISTRY

eventually serving as well as head of department of organic chemistry. The department has three full professors and around 20 graduate students. Teaching languages are French and German. Salary is according to government scale.

Applications with detailed curriculum, a complete list of publications and copies of the most important publications are to be sent before June 15, 1979 to F. P. Emmenegger, dean of the Faculty of Science, University of Fribourg, Pérolles, CH-1700 FRIBOURG, Switzerland.

W97(A)

### PROFESSORSHIP IN MOLECULAR GENETICS

Seeking individual for Professorship in Molecular Genetics. Individual must be established investigator for research and teaching in molecular genetics.

Position in Cancer Centre with broad laboratory research programs including cellular genetics and development, tumor immunology, viral oncology, and chemical carcinogenesis.

Salary negotiable dependent on level of experience and academic rank of applicant. Send curriculum vitae and inquiries to: Chairman, Molecular Genetics Professor Search Committee, Cancer Center/Institute of Cancer Research, Columbia University, 701 West 168th Street, New York, New York 10032.

An Equal Opportunity Employer.

W96(A)

UNIVERSITY OF OTAGO  
Dunedin, New Zealand  
WOLFF HARRIS CHAIR  
OF PHYSIOLOGY

The University Council invites applications from medical or science graduates for appointment to the Wolff Harris Chair of Physiology, at present occupied by Professor J. R. Robinson who is retiring at the end of the year.

Professorial salaries which are regularly reviewed, are paid within the following ranges:—

Medical NZ\$30,365 to NZ\$33,865 per annum.

Science NZ\$23,865 to NZ\$29,865 per annum.

(Both rates inclusive of NZ\$365 per annum cost-of-living allowance).

Further particulars are available from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University.

Applications close on June 30, 1979

611(A)

MEMORIAL UNIVERSITY  
OF NEWFOUNDLAND  
Canada

### POSTDOCTORAL FELLOW DEPARTMENT OF BIOCHEMISTRY

Applications are invited, with two letters of reference for the position of Postdoctoral Fellow to work on the structure of fish antifreeze proteins mechanism of action, biosynthesis and the control mechanism of these proteins, effective April 1, 1979, under the terms of M.R.C. of Canada Salary is \$12,050 first year, \$13,350 second year. The above research will be carried out at the Marine Science Research Laboratory. Write to Dr Choy-L. Hew, Department of Biochemistry, Memorial University of Newfoundland, St John's, Newfoundland A1B 3X9, Canada.

612(A)



## British Museum (Natural History) London

## Entomologists

The British Museum (Natural History) is an institution for taxonomic research and an educational focal point for the public. The main aim of the research is to produce definitive accounts of the world's animals, plants and minerals as well as the presentation of an extensive collection of fossils. These posts are in the Department of Entomology which contains the largest and most comprehensive collection of insects in the world and offers successful candidates unrivalled research opportunities and facilities.

**Medical Insect Studies**

The successful candidate will supervise and participate in taxonomic research on one or more families of medically important Diptera and become a specialist adviser on mosquitoes. The work will also involve improving and maintaining the national collection of medical insects.

Candidates, normally aged under 32, should have a good honours degree or equivalent in zoology, preferably with entomology. At least 2 years' relevant postgraduate experience is essential.

Appointment as Senior Scientific Officer (£5,675 to £7,420) or Higher Scientific Officer (£4,620 to £5,970) according to qualifications and experience. **Salaries under review.** Promotion prospects. Non-contributory pension scheme.

**Taxonomic Research on Coccoidea**

The successful candidate will participate in taxonomic research studies on scale insects and mealy bugs, and maintain and improve the Department's collection through exchanges with other specialists and by collecting and rearing. The work will involve a great deal of microscope slide preparation, and some supervision of junior staff. In addition, the entomologist will deal with enquiries from other institutions and members of the public.

Candidates, normally aged under 30, should have a good honours degree or equivalent in zoology, preferably with entomology. Relevant postgraduate experience is advantageous; at least 2 years is essential for Higher Scientific Officer level.

Appointment as Higher Scientific Officer (£4,620 to £5,970) or Scientific Officer (£3,360 to £4,940) with starting salary according to qualifications and experience. **Salaries under review.** Promotion prospects. Non-contributory pension scheme.

For further details and an application form (to be returned by May 31, 1979) write to Civil Service Commission, Alencon Link, Basingtoke, Hants RG21 1JB, or telephone Basingtoke (0256) 68551 (answering service operates outside office hours). **Please quote ref: SB/54/DK.**

597(A)

AGRICULTURAL  
RESEARCH COUNCILWeed Research  
OrganizationDEVELOPMENTAL  
BOTANY GROUP

## SCIENTIFIC OFFICER

Applications are invited for a Scientific Officer to assist in a research team led by Dr Daphne J. Osborne, which is engaged in physiological, biochemical and ultrastructural studies relating to crop-weed associations.

These include cellular events in seed dormancy, viability and germination, effects of stress, control of meristem growth and shedding of plant parts.

The duties of the post will be mainly concerned with electron microscopy.

Candidates should possess an honours degree, H.N.C. or equivalent in a biological subject with relevant post qualifying experience.

Appointment as Scientific Officer in a scale rising from £2,839 to £4,415 by 12 annual increments. Starting salary will depend on qualifications and experience.

Further particulars and application forms from the Secretary, A.R.C. Weed Research Organization, Begbroke Hill, Yarnton, Oxford OX5 1PF, quoting Ref. 13/79. Closing date May 18, 1979. 643(A)

THE UNIVERSITY  
OF LEEDSDepartment of Inorganic  
and Structural  
Chemistry

Applications are invited for a temporary post of POST-DOCTORAL RESEARCH FELLOW in the above Department from inorganic or biochemists. Candidates should already possess, or expect to receive within the next few months, a Ph.D. degree. Experience in mechanistic studies would be valuable. The appointee will take part in a project, supported by S.R.C., on electron transfer reactions of blue copper proteins, iron-sulphur proteins and cytochrome c. The appointment will be for a fixed period of either one or two years commencing October 1, 1979.

Starting salary in the range £3,883 to £4,382 on the IA scale for Research and Analogous Staff (£3,883 to £6,555) (under review), according to age, qualifications and experience.

Informal enquiries may be addressed in the first instance to Dr A. G. Sykes, Department of Inorganic and Structural Chemistry (Tel: 0532-31751, ext. 6068).

Applications (quoting reference number 44/6/D) should be made as soon as possible to the Registrar, The University, Leeds LS2 9JT (from whom further particulars may be obtained). 641(A)

University of London

British Postgraduate Medical Federation

CARDIOTHORACIC INSTITUTE

(associated with the National Heart &amp; Chest Hospitals)

CARDIAC METABOLISM RESEARCH

LABORATORIES

50 Wimpole Street, London, W.1

A vacancy exists for a

## RESEARCH ASSISTANT

to assist in a project concerned with the identification and mechanism of beta-receptors in myocardial tissue. The project is suitable for a Ph.D. thesis and registration for this higher degree will be encouraged. Applicants should have a degree in Biochemistry (first or upper second class). Those graduating in 1979 may also apply. The post is offered for a maximum of three years. Starting salary £3,615 p.a. Application forms available on request from the House Secretary, Cardiothoracic Institute, 2 Beaumont Street, London W.1 (telephone 01-486 3043). Closing date June 1, 1979.

(This is a readvertisement and previous applicants should not re-apply.) 665(A)



## CENTRAL ELECTRICITY RESEARCH LABORATORIES

Kelvin Avenue, Leatherhead, Surrey, KT22 7SE

# RESEARCH OFFICER

## BIOLOGY SECTION

The Biology Section at CERL, whose research programme includes studies of the environmental consequences of emissions from power stations, requires a graduate/post-graduate environmental scientist to undertake research on certain aspects of the effects of gaseous and particulate emissions ( $\text{SO}_2$ ,  $\text{NO}_x$ , acid precipitation and trace elements) on terrestrial ecosystems. The deposition, uptake and cycling of these atmospheric species in crops and soils are being studied as part of a programme to assess beneficial and detrimental effects of air pollution on crop yield, physiological and biochemical processes in plants, and leaching processes in soils.

The successful candidate will co-operate with physicists and chemists at CERL in the application of information about dispersion and deposition of emissions from power stations and in the development of techniques for studying effects on crop growth, involving measurements of the flux of emissions to and cycling within crops, and their subsequent metabolic fate. Initially, the successful candidate will be particularly involved in micrometeorological and flux measurements associated with the development of systems for fumigating crops both in growth chambers and in the field.

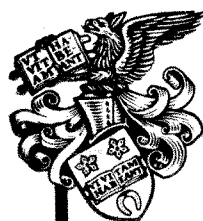
Candidates should have an interdisciplinary background including a good Honours degree in Biology, Physics or Chemistry and preferably postgraduate experience in environmental science or meteorology.

**According to age, experience and qualifications the appointment will be made within incremental salary ranges rising to £6035 or £7050 plus a payment under a Self-Financing Productivity Scheme ranging between £8 and £17 per month.**

The Laboratories are situated in a pleasant part of Surrey and offer attractive conditions of service, and facilities for the total of 800 Research and Support staff engaged in a broad spectrum of research into the materials, technologies and plant performance problems of the Central Electricity Generating Board.

Application forms are obtainable from the Head of Personnel Development & Services Section, Central Electricity Research Laboratories, at the above address or telephone Leatherhead 74488 Ext 363 quoting reference number **RL/30/N**. Closing date is: **Friday 18 May 1979.**

618(A)



## LEICESTER UNIVERSITY

### F. W. BENNETT CHAIR OF GEOLOGY

Applications are invited for the F. W. Bennett Chair of Geology formerly held by the late Professor P. C. Sylvester-Bradley. There is no restriction as to the special interests of applicants.

Further particulars from the Registrar to whom applications should be sent by June 15, 1979. Candidates in the U.K. should submit fifteen copies of their application (overseas candidates may submit one copy).

669(A)

### ANIMAL HEALTH TRUST SMALL ANIMALS CENTRE RESEARCH ASSISTANT

to work on Duck Virus Hepatitis and related duck diseases. The successful applicant will be expected to work with cell culture and virological procedures including serum neutralisation and immunofluorescence. An interest in pathology would be an advantage.

The post will be suitable for either a postdoctoral candidate or a graduate with relevant research experience. Encouragement will be given to register for a higher degree where appropriate.

The initial appointment will be for three years. Initial salary up to £4,500 (with FSSU/USS) according to age and experience.

For further information please contact Dr P. R. Woolcock at the Animal Health Trust, Small Animals Centre, Lanwades Park, Kennett, Newmarket, Suffolk CB8 7PN. Telephone: Newmarket (0638) 750543. Applications to include full curriculum vitae and the names of two referees to be sent to The Director, Animal Health Trust, Lanwades Hall, Kennett, Newmarket CB8 7PN, by June 1, 1979.

675(A)

### MEDICAL RESEARCH COUNCIL LABORATORY ANIMALS CENTRE RESEARCH OFFICER

The Director's research programme is primarily concerned with an evaluation of the advantages which accrue from the use in research of specific pathogen free animals, in particular the cat. A person in the Research Officer category is required to assist the Director in this programme. Opportunities will occur for collaborative work with other departments in the Centre, which cover the whole spectrum of Laboratory animal science.

The Successful applicant will have a degree or equivalent in a biological science, and at least two years relevant research experience. Salary will be related to age, qualification and experience and is supplemented by £354 London Weighting. Contributory pension scheme.

Applications, together with a copy of curriculum vitae and the names of two professional referees, should be addressed to The Director, MRC Laboratory Animals Centre, Woodmansterne Road, Carshalton, Surrey, SM5 4EF. 561(A)

# MRC

Medical Research Council

### UNIVERSITY OF SOKOTO, NIGERIA FACULTY OF SCIENCE

Vacancies exist as follows:

#### 1. DEPARTMENT OF MATHEMATICS:

##### SENIOR LECTURERS LECTURERS

Foundation of Mathematics, Abstract Algebra, Probability Theory and Information Theory, Theoretical Mechanics.

#### 2. DEPARTMENT OF PHYSICS:

##### for PROFESSOR, SENIOR LECTURER

##### and ASSISTANT LECTURER

**SALARIES:** Professor, N11,268 to N12,420; Senior Lecturer, N7,764 to N8,724; Lecturer I, N7,104 to N7,752; Lecturer II, N5,460 to N6,432; Assistant Lecturer, N4,368 to N5,340 N.B.: N1=approx. 79p.

Interested candidates should apply for further details to:

Principal Assistant Secretary  
(Recruitment)  
Nigerian Universities Office  
180 Tottenham Court Road  
London W1P 9LE

661(A)

### DEPARTMENT OF BIOPHYSICS King's College London POSTDOCTORAL RESEARCH ASSISTANT

Postdoctoral position supported by the M.R.C. is available from June 1, 1979, to collaborate with Dr H. J. Gould on the molecular cloning and analysis of human immunoglobulin genes.

Starting date not later than September 1, 1979. Salary £3,883 to £6,555 p.a. plus London weighting.

Applications, with curriculum vitae and names of two referees, should be sent to Dr H. J. Gould, Department of Biophysics, King's College, 26-29 Drury Lane, London WC2B 5RL. 626(A)

# EUROPEAN FIELD TRIALS MANAGER

The Agricultural Products Research and Development Division of Pfizer Central Research at Sandwich, Kent, is expanding its capability to conduct opinion leader and field trials throughout Europe (including U.K.) with animal health and performance enhancing products.

Applications are invited for the new position of European Field Trials Manager whose work will embrace both the planning and personal supervision of such field trial programmes in the broad animal health products area.

His or her work will cover both development projects with new product candidates and market support programmes designed to extend the applications and acceptance of existing Company products.

The technical spheres involved include animal performance enhancement in its several guises and the broad anti-infective range of antibacterials, anthelmintics and ectoparasitides.

The successful candidate will work closely with R and D colleagues in relevant scientific disciplines and departments, particularly at the planning stage, and will personally implement agreed trial programmes in the field. He or she will be based at Sandwich, and will spend some 50 per cent of working time in the field, mostly in Continental Europe.

We are seeking a Veterinarian or other suitably qualified individual in the late 20s to early 40s age range, with drug development field trials experience, fluency in at least two European languages (one of them English) and, preferably, existing appropriate field contacts for trial work on commercial premises and at 'expert' locations.

As well as technical suitability, initiative and enthusiasm, the personal qualities required are those appropriate to effective interaction with a variety of collaborators in Europe both within

and outside the organisation, and the overall reliability and ambassadorship essential to operating in part away from home base.

This post will attract a high salary, reflecting both the breadth of responsibility and geographical scope of the duties. A car will be provided. The Company is situated in rural and coastal Kent with many local recreational amenities. We offer generous conditions of employment including bonus, pension, death benefit and salary continuance schemes, flexible working hours and an excellent Sports and Social Club.

This new appointment is an important one in Pfizer's expanding programme of Agricultural Products Research and Development. If you have the qualifications, qualities and experience necessary to make a significant contribution to our operations, please send a brief curriculum vitae, which will be treated in the strictest confidence, to:

**Pfizer**

**R. G. MULHERN, Director, Research Personnel, Pfizer Central Research, Ramsgate Road, SANDWICH, Kent CT13 9NJ or telephone 03046 3511 extension 241 for an application form.**

603(A)

## BRUNEL UNIVERSITY LECTURESHIPS IN THE SCHOOL OF BIOLOGICAL SCIENCES

1. Applications are invited for a lectureship in the Department of Applied Biology. An interest in teaching and research in the general field of Eukaryote Genetics will be a decided advantage.
1. Applications are invited for a lectureship in the Department of Biochemistry.

The salary for both posts will be on the Lecturer scale £3,883 to £7,754 plus £502 London Allowance.

Write for application form and further details for both posts to the Assistant Secretary (Establishment), Brunel University, Uxbridge, Middlesex UB8 3PH or telephone Uxbridge 37188 extension 49. Closing date: May 18, 1979.

THE UNIVERSITY OF MANCHESTER. Department of Medical Biochemistry. 1. RESEARCH TECHNICIAN (Grade 5) required to work on the biochemistry of connective tissues. This position, financed by the Arthritis and Rheumatism Council, is available to the end of March 1981. Applicants should have H.N.C. or equivalent and at least 8 years experience in biochemistry. Familiarity with tissue culture techniques would be an advantage. Salary scale £3,474 to £4,056 per annum. 2. TECHNICIAN (Grade 4) required for a variety of tasks. Candidates should have an O.N.C. and several years relevant laboratory experience. A knowledge of centrifugation would be an advantage. Salary scale £3,222 to £3,708 per annum. Applications for these posts (please specify which) should include details of age, experience and qualifications and the name and address of two referees and be sent to the Secretary, Department of Medical Biochemistry, University of Manchester Medical School, Manchester M13 9PT. 668(A)

## SCIENTIST

### to work on MOTION SICKNESS

at the Institute of Naval Medicine, Alverstoke, Hants, with support administered by the MEDICAL RESEARCH COUNCIL in a 3 year project. The appointee will be of postdoctoral status preferably with some experience in physiology and/or pharmacology. Medical qualifications and/or experience of working with human subjects advantageous.

Salary according to qualifications, experience and age, in the range; £4,600 to £6,500.

Further particulars from the Assistant Secretary, Royal Naval Personnel Research Committee, Room 407, First Avenue House, 40-48 High Holborn, London WC1V 6HE.

**MRC**

Medical Research Council  
667(A)



# Synthetic Organic Chemistry

Hoechst – one of the largest pharmaceutical companies in the world, with an active international research and development programme – are expanding their drug discovery programme at their research laboratories at Milton Keynes, Bucks, and are seeking a number of organic chemists to join project teams within the department of synthetic organic chemistry.

Candidates should have a real interest in all aspects of synthetic chemistry and be able to apply their innovative and practical skills to medicinal chemistry and the syntheses of compounds for biological testing. For these challenging new posts applications are invited from:

PhD Chemists with up to two years' post-doctoral experience in synthetic organic chemistry.

Graduates (BSc, GRIC) with up to three years' relevant industrial experience.

People who expect to graduate with a relevant first or higher degree this year.

These appointments carry good salaries, with at least four weeks holiday a year. Benefits include flexible working hours, free private health scheme, subsidised restaurant meals, excellent sports and social facilities. Generous relocation assistance will be provided if applicable.

Please write or telephone for an application form to:  
Mr. A. Forrest, Personnel Department, Hoechst UK Limited,  
Walton Manor, Walton, Milton Keynes, Bucks. Tel: Pineham 5068.

## Hoechst



637(A)

## THE DISTILLERS COMPANY LIMITED RESEARCH CHEMIST

We have a vacancy for an honours graduate in chemistry, aged 24 to 29, with experience in chromatographic methods, preferably as applied in food, beverage or flavour analysis. The successful candidate will be expected to develop methods of analysis for constituents of portable spirits and will have the best facilities to adopt his/her analytical expertise to solving problems which arise in spirit manufacture.

The company runs well equipped laboratories in pleasant surroundings. A non-contributory pension scheme is operated and assistance can be given with removal expenses.

For further information and an application form write, with brief details of career to: Dr R. E. B. Duncan, Manager, The Distillers Company Limited, Glenochil Research Station, MENSTRIE, Clackmannanshire. Tel: Alva (0259) 61481.

678(A)

## UNIVERSITY OF MANCHESTER DEPARTMENT OF PHYSICS RESEARCH ASSISTANT

Applications are invited for the above post, supported by the SRC under an agreement with the Daresbury Laboratory. The successful applicant will be responsible for the commissioning of a large NaI total-energy  $\gamma$ -ray spectrometer and for the construction and testing of gas ionization and gas scintillation detectors to be used in the nuclear physics experimental programme at the Daresbury Nuclear Structure Facility. Candidates should have a good honours degree in physics and should possess the necessary attitude for pursuing a programme of apparatus development. Consideration will be given to candidates graduating this summer. The post is tenable for a period ending in April 1981 at a salary in the range £3,384 to £4,882 p.a. Superannuation. Further information is available from Dr J. C. Lisle, The Schuster Laboratory, The University, Manchester, M13 9PL, to whom applications and the names of two referees should be sent by June 1, 1979/N.

604(A)

## MEDICAL RESEARCH COUNCIL LABORATORY ANIMALS CENTRE RESEARCH OFFICER

The Centre's Accreditation Department manages a national scheme concerned with monitoring the quality of laboratory animals of many species produced by commercial breeders and suppliers. The scheme aims to ensure that adequate standards of housing and husbandry are maintained; it acts as an information exchange on sources of supply; and it provides veterinary advice to the centre.

A vacancy in the Research Officer category exists in the department. The post holder will be expected to travel widely in Great Britain. Applications are invited from persons with a degree or equivalent qualification in a biological science, and at least two years research experience. Salary will be related to age, qualification and experience and is supplemented by £354 London Weighting. Contributory pension scheme.

Applications, together with a copy of curriculum vitae and the names of two professional referees, should be addressed to The Director, MRC Laboratory Animals Centre, Woodmansterne Road, Carshalton, Surrey, SM5 4EF. 562(A)

# MRC

Medical Research Council

## UNIVERSITY OF NAIROBI—KENYA

Applications are invited for the following posts in the DEPARTMENT OF BOTANY:—

### SENIOR LECTURER

Applicants should be qualified in any of the following disciplines of Genetics: Molecular Genetics; Population Genetics and Quantitative Genetics. Candidates should also have Ph.D. in Botany with extensive teaching and research experience evidenced by published works. Appointee will be expected to teach undergraduate and postgraduate courses and develop research programmes in their specialism.

### LECTURERS

Applicants should be highly qualified with commitment to teaching and research in any of the following: i) Plant Physiology and Biochemistry with special interest in plant growth and development; ii) Taxonomy (mainly experimental systematics, chemosystematics and plant geography); iii) Microbiology (with interests in mycology and/or microbial genetics); iv) Marine Botany (marine ecology or marine microbiology). Participation in the undergraduate and postgraduate courses and development of research programmes in their areas of specialisation is expected.

Salary scale: Senior Lecturer K£2,988 to K£3,984 per annum, Lecturer K£2,016 to K£3,312 per annum (K£1 equals £1.29 sterling). The British Government may supplement salaries in range £4,278 to £5,430 per annum (sterling) for married appointees and £2,730 to £3,648 per annum (sterling) for single appointees (reviewed annually and normally free from tax) and provide children's education allowances and holiday visit passages. Family passages; superannuation scheme; medical aid scheme; various allowances. Detailed application (two copies) with curriculum vitae and naming three referees to be sent direct to Registrar, University of Nairobi, PO Box 30197, Nairobi, Kenya by May 12, 1979. Applicants resident in the U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address.

608(A)

## UNIVERSITY OF EDINBURGH

### DEPARTMENT OF FORESTRY AND NATURAL RESOURCES

Applications are invited for two  
lecturer positions in

- (i) **FOREST  
MANAGEMENT**
- (ii) **WILDLIFE  
ECOLOGY**

For position (i) applicants should have a University degree in Forestry and some practical experience of Forest Management. Additional experience in a cognate field, such as mensuration, statistics, economics, resource appraisal or wildlife management would be an advantage.

For position (ii) applicants should be ecologists with some practical knowledge of population dynamics and of the physiology of vertebrates. Additional experience in wildlife management would be an advantage.

The successful candidates will become members of a Department covering a wide range of topics in Ecological Science and the management of renewable natural resources and will be expected to cooperate in teaching in integrated ecology courses as well as in the above areas; they will also be expected to pursue, in collaboration with their colleagues, research on relevant topics.

It is hoped that the successful candidates will take up the positions on October 1, 1979. Salary scale: £3,883 to £7,754 per annum (under review).

Applications (three copies) together with the names of two referees should be lodged not later than June 1, 1979 with the Secretary to the University, Old College, South Bridge, Edinburgh EH8 9YL, from whom further particulars may be obtained. Please quote reference 1041. 600(A)

## UNIVERSITY OF CALIFORNIA, BERKELEY

### College of Natural Resources

Faculty and Agricultural Experiment  
Station Position in the Division of  
Biological Control and Department  
of Entomological Sciences

A position as Professor of Entomology and Entomologist in the Experiment Station will be available July 1, 1979. Qualifications must include a Ph.D. degree with specialisation in the biology, ecology, and systematics of parasitoids and predators of arthropod pests, together with extensive kills and 15 years of experience in these areas, and in foreign exploration, importation, colonisation and evaluation of such agents in biological control programs. Research responsibilities (80%) in the Division of Biological Control will include studies in the biology, ecology and behaviour, and on origin exploration, importation, evaluation, augmentation, and conservation of natural enemies of arthropod pests of agricultural crops and urban plantings, and in the cooperative development of integrated pest management programs. Teaching responsibilities (20%) will be in the Department of Entomological Sciences in the area of biological control. Applicants should send full curriculum vitae, relevant publications and manuscripts (official academic transcripts), and the names of three persons as reference by June 1, 1979 to:

L. E. Caltagirone, Chairman  
Division of Biological Control  
University of California  
Albany, California 94706

The University of California is an equal opportunity, affirmative action employer. W85(A)

The Daresbury Laboratory has a vacancy in the Computational Science group for a scientist with experience in

# COMPUTATIONAL ATOMIC AND MOLECULAR COLLISIONS

The position is at Senior Scientific Officer level.

The successful applicant will be responsible for providing general support for collaborative computational projects involving atomic and molecular collisions being conducted in collaboration with the Universities. The work will include the development and maintenance of large computer programs in this area of science. Applicants should possess a Ph.D. in Physics or Chemistry and have relevant postdoctoral experience in computational atomic and molecular collisions.

The computational science group is part of the Division of Theory and Computational Science. The Division has broad-ranging interests in theoretical and computational physics, chemistry and biological sciences. The Laboratory has a purpose built X-ray and U.V. source and a 30 MV tandem Van de Graff accelerator under construction. It also has powerful computing facilities including an IBM 370/165 on site and a link to two IBM 360/195 computers at SRC Rutherford Laboratory.

Appointments will be made at salaries between £5154 and £6898 depending on age, ability and experience and may be taken up on secondment from a University for a period of five years or on a permanent basis.

Closing date: 17 May 1979.

For further information please write or telephone to the head of the computational science group, Dr. V. R. Saunders (Tel: (0925) 65000 Ext. 204). Applications should be sent together with curriculum vitae and the addresses of two referees quoting reference number DL/672/T to:

The Personnel Officer

## DARESBURY LABORATORY

Science Research Council  
Daresbury, Warrington WA4 4AD

617(A)

## UNIVERSITY OF SIERRA LEONE

### Njala University College

Applications are invited for the  
posts of

### PROFESSOR AND LECTURER

in the DEPARTMENT OF BIOLOGICAL SCIENCES tenable as from September 1, 1979. Preference will be given to candidates with teaching and research interests in Microbiology, Immunology and Genetics. Salary scales:—Professor Le8,000 to Le9,240 per annum. Lecturer Le4,488 to Le6,897 per annum (£1 sterling equals Le2.20). The British Government is unlikely to provide salary supplementation and associated benefits. University Superannuation scheme or contract terms; Family passages; annual leave; car loan negotiated. Part-furnished accommodation at reasonable rental; various allowances. Detailed application (two copies) with curriculum vitae and naming three referees, to be sent to the Secretary, University of Sierra Leone, Freetown, Sierra Leone by June 23, 1979. Applicants resident in the U.K. should also send one copy to the Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. 644(A)

## UNIVERSITY OF THE WEST INDIES—JAMAICA

Applications are invited for the  
post of

### LECTURER/ASSISTANT LECTURER

in the DEPARTMENT OF BOTANY. The appointee will be required to teach Botany to B.Sc. level. Preference may be given to applicants with interest and teaching experience in Agricultural Microbiology, Forestry or Horticulture. Salary Scale: (under review) Lecturer J\$8,913 to J\$13,917 per annum. Assistant Lecturer J\$7,236 to J\$7,926 per annum (£1 sterling equals J\$3.62). Family passages; F.S.S.U.; Study and Travel Grant. Unfurnished accommodation will be let by the University at a rental of 10 per cent of salary or a housing allowance of 20 per cent of salary is payable. Detailed applications (three copies) with curriculum vitae and naming three referees should be sent direct, as soon as possible, to the Registrar, University of the West Indies, Mona, Kingston 7, Jamaica. Applicants resident in the U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 633(A)

## CITY OF LONDON POLYTECHNIC

A post of

### RESEARCH ASSISTANT

for studies leading to a higher degree has fallen vacant in the Department of Geology. Candidates who might wish to carry out research in one of the following projects should apply in writing giving full curriculum vitae and the names and addresses of two referees to the Staff Records Officer, City of London Polytechnic, 117 Houndsditch, London EC3A 7BU quoting reference number 79/34.

(a) Geology of the nappe region of North-east Jotunheim, Norway.

(b) Volcanology of the Tayvallich Lavas and associated Igneous rocks of S. W. Argyll.

(c) The geological evolution of the Pre-cambrian Stora Le Marstrand Series of Northern Orust, Sweden.

The salary scale for Research Assistants is £2,613 to £2,700 to £2,787 plus London Allowance of £474 per annum. 660(A)

## Northern Ireland Forensic Science Laboratory

### Scientific Staff

Applications are invited for the following pensionable posts in the Northern Ireland Forensic Science Laboratory, Newtownbreda Road, Belfast.

The successful candidates will join a team of scientists engaged in the scientific investigations of crime throughout Northern Ireland. The work is interesting and varied and offers wide scope for developing interests in the varied aspects of forensic science.

#### Scientific Officers

**£2,839 – £4,415 (Ref: SB 189/79/N)**

Applicants must be under 27 years of age at December 31, 1979 and possess

- (a) an Honours degree in pure or analytical chemistry; or
- (b) an equivalent qualification acceptable to the Civil Service Commissioners.

Applicants who hope to obtain the requisite qualification in 1979 may apply.

#### Metallurgist

**(Higher Scientific Officer)**

**£4,101 – £5,448 (Ref: SB 109/79/NN)**

Applicants must be under 30 years of age at December 31, and possess

- (a) a 1st or 2nd class Honours degree in Metallurgy; or
- (b) an equivalent qualification acceptable to the Civil Service Commissioners.

Candidates must have at least 2 years' postgraduate experience in research or industry.

Exceptionally, applications may be considered from candidates over the age limits who have specialised experience.

Starting salary will be related to qualifications and experience.

The salary scale is under review with effect from April 1, 1979.

There are promotion prospects to Higher Scientific Officer £4,101 to £5,488, Senior Scientific Officer £5,154 to £6,898 and Principal Scientific Officer £6,609 to £8,461.

Please write or telephone for an application form quoting the appropriate job reference to the Civil Service Commission, Rosepark House, Upper Newtownards Road, Belfast BT4 3NR (telephone Dundonald 4585 ext 256). Completed forms must be returned to arrive not later than May 18, 1979. 652(A)

## UNIVERSITY COLLEGE LONDON

### Applications invited for post of RESEARCH ASSISTANT

to work on problems of microtubule assembly and its relationship to spindle formation and mitosis in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* under the supervision of Dr J. S. Hyams. Appointment will be for three years and successful applicant may register for a higher degree. Applicants should hold, or expect to obtain, a first or upper second class honours degree in Microbiology, Cell Biology, Biochemistry or related subject. Starting salary £3,410 per annum (under review) plus £502 London Allowance. Applications in form of curriculum vitae, giving names and addresses of two referees to Dr J. S. Hyams, Department of Botany and Microbiology, University College London, Gower Street, London WC1E 6BT, from whom further details may be obtained. Closing date May 24, 1979. 636(A)

## M. D. ANDERSON PROFESSOR IN PHYSICS

The University of Houston is seeking candidates for an M. D. Anderson Professor, an endowed chair, in the Department of Physics. The applicants should be established leaders in their field of physics. The successful candidate is expected to provide strong and constructive intellectual leadership not only to the Physics Department but also to the institution as a whole. General duties of this appointment will include both teaching and research.

The current departmental areas of research consist of condensed matter, intermediate energy, plasma, space, and theoretical physics.

Applicants should include a resume, a brief description of professional interests and goals, and the names and addresses of three or more references. Send applications to: C. W. Chu, Chairman, Search Committee, Department of Physics, University of Houston, Houston TX 77004. The University of Houston is an equal opportunity employer. W91(A)

## NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING

(UNIVERSITY OF READING)

### NUTRITION DEPARTMENT

A graduate is required to participate in a programme of fundamental research into the physiological role of trace nutrient binders in milk and other foods. The work will be mainly concerned with the isolation, characterisation and comparative biochemistry of binding substances from milks of different mammalian species, in particular from bovine and human milk. The appointed person will work within a small group studying binding substances and will be expected to initiate and execute experimental work with a minimum of supervision.

Qualifications: First or Upper Second class Honours Degree in biochemistry or recently qualified Ph.D. Applicants with experience in protein chemistry and enzymology will be at an advantage.

Appointment will be as Scientific Officer (£2,839 to £4,415) or Higher Scientific Officer (£4,101 to £5,448) according to qualifications and experience. At least two years' relevant postgraduate experience is required for appointment as H.S.O. Non-contributory superannuation scheme.

Application forms are obtainable from the Secretary, NIRD, Shinfield, Reading RG2 9AT. Quote reference 79/15. 595(A)

## UNIVERSITY OF MALAYA

Applications are invited for the posts of

### LECTURER

in the following departments in the Faculty of Science:

#### DEPARTMENT OF BOTANY

Candidates must possess a higher degree in the field of Botany or Agriculture. Preference will be given to candidates who have a Ph.D. degree and experience in Tropical Crops.

#### DEPARTMENT OF GEOLOGY

In the field of Structural Geology/Rock Mechanics.

#### DEAN'S ESTABLISHMENT

The fields required are as follows: Scientific Method, Scientific teaching, logic, history of science in relation to Greek Science, Chinese Science, Islamic Science and Modern Science and philosophy of Science.

#### QUALIFICATIONS

Candidates for the appointments should possess at least: A Master's degree in the required field; or a Ph.D. in the required field.

**Salary Scale** (All inclusive): £3,602 by 168 to 3,939/4,107 by 168 to 4,443/4,780 by 280 to 6,182/Review Point/£6,462 by 336 to 6,799 per annum. The commencing salary for Lecturers with a Ph.D. degree shall be £4,107 per annum.

Further particulars and application forms are obtainable from the Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF.

The closing date for the receipt of applications is **May 31, 1979.** 664(A)

## AN DER FREIEN UNIVERSITÄT BERLIN IST FOLGENDE STELLE ZU BESETZEN

*Fachbereich 1—Natur- und sozialwissenschaftliche Grundlagenmedizin und medizinische Ökologie—Institut für Anatomie*

### PROFESSOR (C 4)

**Aufgabengebiet:** Vertretung des Faches Anatomie in Forschung und Lehre. Wissenschaftliche Arbeitsmöglichkeiten auf allen morphologischen Teilgebieten stehen zu Verfügung. **Einstellungsvoraussetzung:** \$ 13 BerIHG. Bewerbungen mit den üblichen Unterlagen sind innerhalb von 6 Wochen zu richten an den Fachbereich 1—Natur- und sozialwissenschaftliche Grundlagenmedizin und medizinische Ökologie—Gustav Meyer-Strasse 7, 1000 Berlin 33. W92(A)

## UNIVERSITY OF BRISTOL DEPARTMENT OF PHYSIOLOGY POSTGRADUATE RESEARCH ASSISTANT

to work within a small research group on cellular neurobiology of sensor and motor systems in crustaceans employing electrophysiological, pharmacological and computer techniques. The position is available for up to two years, at a starting salary of £3,38 per annum (scale under review).

Applications with curriculum vitae and names of two referees to Dr B. M. H. Bush, Department of Physiology, The University, Bristol BS8 1TD, by May 25, 1979. 599(A)

## Scientific Officer £2,839 – £4,415

Applications are invited for a pensionable post of Scientific Officer in the Department of Commerce, Industrial Science Division, which is located at 17 Antrim Road, Lisburn.

The successful candidate will assist in developing the Information Service of a Division which provides a wide range of technical services in Northern Ireland for Industry and Government. The duties will include answering technical enquiries extending over a wide field, particularly from industry; compilation of literature surveys, and provision of current awareness services. There will be particular emphasis on the use and application of computerised methods for information retrieval.

Applicants must be under 27 years of age on December 31, 1979 and have a degree, HNC/HND, or equivalent qualifications in chemistry or physics. Those hoping to qualify in the summer graduations may apply. A qualification in Information Science, or experience of technical information work and an aptitude for languages will be an advantage.

Exceptionally applications may be considered from candidates over the age limits who have specialised experience.

The Commissioners may decide to interview only those applicants who appear from the information available (including level of academic attainments and experience) to be best qualified.

There are promotion prospects to Higher Scientific Officer (£4,101–£5,448) Senior Scientific Officer (£5,154–£6,898) and Principal Scientific Officer (£6,609–£8,461).

Starting salary will be related to qualifications and experience.

The salary scale is under review with effect from April 1, 1979.

Please write or telephone for an application form quoting job reference SB 200/79/N to the Civil Service Commission, Rosepark House, Upper Newtownards Road, Belfast BT4 3NR (telephone Dundonald 4585 ext 256). Completed forms must be returned to arrive not later than May 17, 1979.

642(A)



**NORTHERN IRELAND  
CIVIL SERVICE**

## UNIVERSITY OF SIERRA LEONE

NJALA UNIVERSITY COLLEGE

Applications are invited for the post of

### LECTURER IN PHYSICAL CHEMISTRY

tenable from September 1, 1979. Candidates are required to have a good first degree in Chemistry and research and teaching experience in Physical Chemistry. The appointee will be required to teach Physical and General Chemistry up to B.Sc. General degree level; to instigate and supervise student projects, and participate in research within the Department. Salary scale:— Le4,488 to Le6,897 per annum. (£1 sterling = Le2.20). The British Government is unlikely to provide salary supplementation and associated benefits. Family passages; University Superannuation scheme or contract terms; annual leave; car loan negotiated; various allowances; part-furnished accommodation at reasonable rent. Detailed applications (2 copies) with curriculum vitae and naming three referees to be sent to Secretary, University of Sierra Leone, Private Mail Bag, Freetown, Sierra Leone, by June 23, 1979. Applicants resident in the UK should also send one copy to the Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address.

670(A)

## UNIVERSITY OF DURHAM CHAIR IN GEOLOGY

Applications are invited for the from candidates with research interests in any of the main fields of Geology. The appointment will date from October 1, 1979 or such later date as may be arranged.

The vacancy arises from the appointment of Professor G. M. Brown F.R.S. as Director of the Institute of Geological Sciences.

The appointment will be made on the Professorial salary scale together with the usual pension arrangements.

Applications (three copies), including the names of three referees, must be submitted not later than June 5, 1979 to the Registrar and Secretary, Old Shire Hall, Durham DH1 3HP, from whom further particulars may be obtained.

605(A)

A position is available in the Department of Biochemistry, University of Alberta, Edmonton, T6G 2H7, beginning in the fall of 1979. Applicants should have a good background in DNA metabolism and DNA-drug interactions, both at the graduate and postdoctoral level. A strong background in physicochemical techniques is required for current studies on multistranded nucleic acids and their possible biological roles. Only Canadian Citizens may apply for this position. Please apply to Dr A. R. Morgan at the above address.

W73(A)

# Laser Scientists. Plasma Physics.

You will be joining a small experimental team to provide facilities for and collaborate with university users of the SRC Central Laser Facility. The experiments are primarily in laser driven implosion and compression of plasmas and laser plasma interactions. The work involves principally the development and maintenance of new measurement systems of an electronic or opto-electronic nature as well as assisting University research workers with the operation of the laser target facilities.

You should have a university degree in a relevant subject. Experience in pulse electronics, automated data recording or optics would be advantageous.

## Glass Laser Development.

You will be a member of a team that is responsible for the development and operation of the neodymium glass laser at the SRC Central Laser Facility. The work is mainly experimental and is associated with areas such as the development of improved laser oscillators, the investigation of pulse shaping techniques and the design of laser diagnostics. This post offers scope for an individual contribution in an area of applied physics where a sound knowledge of optics and laser physics is particularly appropriate.

You should have a university degree in a relevant subject.

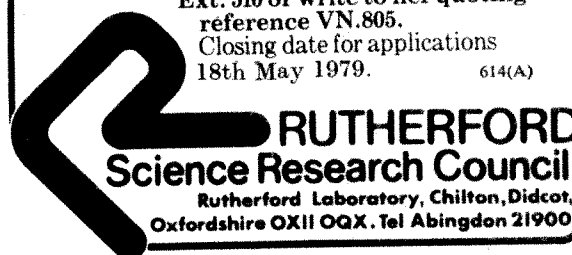
The appointments will be made to the Scientific Officer grade and depending on qualifications and experience, salaries will be within the range £3,037-£4,724. Salaries are currently under review.

Contact Jane Griffiths in the Personnel Group on Abingdon (0235) 21900

Ext. 510 or write to her quoting reference VN.805.

Closing date for applications 18th May 1979.

614(A)



**RUTHERFORD  
Science Research Council**

Rutherford Laboratory, Chilton, Didcot,  
Oxfordshire OX11 0QX. Tel Abingdon 21900

## UNIVERSITY OF CAMBRIDGE DEPARTMENT OF PATHOLOGY

Applications are invited for a postdoctoral

### RESEARCH ASSISTANT (aged under 30)

on a project funded by the M.R.C. for an 8 month period to work on the Genetics of the Immune Response and in Tissue Culture Methods. Salary according to age. Applications in writing to The Superintendent, Department of Pathology, Tennis Court Road, Cambridge CB2 1QP.

627(A)

## Applied Mathematics and Theoretical Physics CAMBRIDGE UNIVERSITY

Applications invited for an Assistant Lectureship vacant on October 1, 1979. No restriction on research field, but strong applications from specialists in mechanics of solid materials are especially welcome. Teaching duties include giving two courses of lectures. Standard university salary scale. Appointment for three years initially. Further information available from Head of Department of Applied Mathematics and Theoretical Physics, Silver Street, Cambridge CB3 9EW. Applications required by May 31, 1979.

658(A)





**The POLYTECHNIC  
WOLVERHAMPTON**

# Research Assistants

Applications are invited for posts available for work on the following projects from 1 September 1979 or for work on a project specified by the applicant in one of the following areas of study:

## SOCIAL SCIENCE

1. The Psychology of Stuttering.
2. How Personality Factors Influence Role-taking and Outcome of Experimental Groups.

## SCIENCE

3. Educational Management Information Systems.
4. Function of Peroxisomes in Fat Metabolising Cells of Animals.
5. Hot Corrosion of Alloys in Coal-Fired Fluidised Combustion.

## ENGINEERING

6. The Development of a High-Strength Tool Geometry.
7. Optimum Machining Conditions for Reaction-Bonded Silicon Carbide.
8. Heat Transfer and Vibration in Heat Exchangers.

Appointments will be made in June and are normally for two or three years. Successful applicants will be expected to register for a higher degree where appropriate.

Salary from £3000 per annum.

Further details and application form (returnable by 7th June) from:  
'Personnel' (RA3), The Polytechnic,  
Wulfruna Street, Wolverhampton, WV1 1LY.  
(Telephone: 0902 27371 Ext. 94)

## IMPERIAL COLLEGE

### RESEARCH PHYSICIST

Applications are invited for a tenured post in High Energy Nuclear Physics Group of the Department of Physics. The successful candidate will be involved in particle physics research and will guide equipment development and design. This will necessitate working with both the Counter and Bubble Chamber sections of the Group and could involve measuring machine development, development of hybrid hardware for triggered bubble chamber experiments or development of detectors. Candidates with a research background outside particle physics will be considered. Starting salary will be on the research and analogous 1A scale £4,760 to £7,647 (including London allowance) according to suitability. Closing date May, 28 1979. Please apply to:

T. W. Dickson,  
Blackett Laboratory,  
Imperial College,  
London SW7 2AZ.

654(A)

## VETERINARY PATHOLOGISTS

Hazleton Laboratories, one of Europe's leading laboratories serving the Safety Evaluation needs of the chemical and pharmaceutical industries, require veterinarians experienced or interested in the rapidly developing field of laboratory animal pathology.

Experienced candidates would be expected to have at least 2-3 years experience in pathology, preferably in a commercial appointment. New graduates or post-graduates without previous experience in Pathology would be given suitable in-house training and encouraged to attend outside courses and seminars.

Based in Harrogate within easy access of the Yorkshire Dales, the posts carry generous remuneration, assistance with re-location, and other fringe benefits to be expected from an expanding and progressive company.

To find out more about the posts, the company and Harrogate please telephone:

Barbara Grant  
Hazleton Laboratories Europe Limited  
Otley Road  
HARROGATE.

Telephone Harrogate (0423) 67265.

615(A)



**HAZLETON**  
LABORATORIES EUROPE LTD

## UNIVERSITY OF LONDON

### KING'S COLLEGE SCHOOL OF HUMAN

### ENVIRONMENTAL STUDIES

Applications are invited for a

### LECTURESHIP

in Human Environmental Studies. The applicants should have a knowledge of geomorphology, climatology and other aspects of physical geography and be able to relate these to environmental issues. Cartographic and statistical qualifications are also required and a background in ecology would be advantageous.

Current salary scale £3,883 to £7,754 per annum (under review) plus £502 per annum London Allowance. The starting salary will be at an appropriate point on the scale. Universities Superannuation Scheme contributions would be payable.

Application forms and further particulars are available from the Assistant Registrar, King's College London, Strand, LONDON WC2R 2LS and should be returned to him in triplicate to reach him no later than May 15, quoting ref. 191675 N.

606(A)

## ROYAL POSTGRADUATE

### MEDICAL SCHOOL ENDOCRINE UNIT

### RESEARCH OFFICER

required for studies on the regulation of Vitamin D metabolism. Work will involve cell culture, chromatography studies and in vivo experiments.

Applicants must have or expect to be awarded a first class or upper second class honours degree in an appropriate subject. There may be an opportunity for Ph.D. registration for a suitable candidate.

Starting salary £3,410 plus £45 London Allowance rising on scale 1 for Research and Analogous Staff Scales currently under review. The appointment is for three years supported by the Medical Research Council.

For further details and an informal discussion please phone Professor MacIntyre on 01-743 2030, extension 457.

Application forms may be obtained from the Personnel Office, Royal Postgraduate Medical School, 150 D Cane Road, London W12 0HS quoting reference number 4/210/N.

613(A)

## UNIVERSITY OF SOUTHAMPTON

### MEDICAL ONCOLOGY UNIT

Applications are invited for the position of Postdoctoral Research Fellow in the above Unit. The person appointed will work on the isolation of cytoskeletal proteins and their interaction with the cell surface, in close cooperation with a group interested in cell adhesion and with others interested in clinical aspects of cancer research. Applicants should either be about to obtain or have recently obtained a Ph.D. in Biochemistry or some related discipline. Experience of protein biochemistry, membrane biochemistry or immunology would be advantageous. The appointment will be for one year in the first instance, renewable annually for at least three years.

Salary Range: £3,883 to £4,822 per annum (under review). U.S.S. benefits.

Applications giving date of birth, curriculum vitae and the names and addresses of two referees, should be sent to Mrs P. Vaughan-Smith, The University, Southampton SO9 5NH, not later than May 18, 1979. Please quote reference 1069/R/N.

598(A)

# UNIVERSITY OF NAIROBI Kenya

Applications are invited for the following posts in the

## DEPARTMENT OF PHYSICS ASSOCIATE PROFESSOR

Applicants must possess a PhD in Physics with considerable years of University teaching and substantial research experience as evidenced by publications in internationally reputable journals. Active areas of research in the Department include Solid State Physics, Applied Geophysics, Paleomagnetism, Electronics and Theoretical Physics.

### SENIOR LECTURER

Applicants must possess a PhD in Physics with considerable years of University teaching and research experience as evidenced by publications in reputable journals. Specialisation in Applied Geophysics, Solid State Physics, Theoretical Physics, Electronics or Paleomagnetism will be an added advantage.

Salary scales: Associate Professor K£3,864 to £4,488 per annum, Senior Lecturer K£2,988 to £3,984 per annum (K£1=£1.28 sterling). The British Government may supplement salaries in range £5,430 to £5,784 per annum (sterling) for married appointees and £3,648 to £3,648 to £3,876 per annum (sterling) for single appointees (reviewed annually and normally free from tax) and provide children's education allowances and holiday visit passages. Family passages; S.S.S.F. or F.S.S.U.; non-contributory medical scheme; subsidised housing or housing allowance. Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct, to Registrar (Recruitment and Training), University of Nairobi, P. O. Box 30197, Nairobi, Kenya by June 24, 1979. Applicants resident in the UK should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 679(A)

## UNIVERSITY OF OXFORD POSTDOCTORAL RESEARCH ASSISTANT AND TECHNICIAN

required for research into the regulation of branched chain 2-oxo acid dehydrogenase complex, branched chain amino acid catabolism and into disorders of branched-chain amino acid metabolism. The posts are for three years from a mutually convenient date in 1979 and are funded by the Medical Research Council. The postdoctoral research assistant should have experience in enzymology and protein chemistry and possess or expect a Ph.D. or equivalent degree. The technician should have a degree in biochemistry (or a related subject) or possess suitable laboratory experience. Starting salaries up to £4,382 (research assistant) and £3,930 (technician) on scales in operation on March 1, 1979.

Applications including the names of two referees to Professor P. J. Randle, Department of Clinical Biochemistry, Radcliffe Infirmary, Oxford OX2 6HE from whom further details may be obtained. 659(A)

Postdoctoral/Research Associate position available immediately to work on the cloning of soybean leghaemoglobin and other plant genes obligatory for symbiosis with *Rhizobium*. Experience in the area of nucleic acid, biochemistry and microbiology desired. Salary commensurate with experience. Send résumé and letters of reference to Dr D. P. S. Verma, Department of Biology, McGill University, Montreal, Quebec, Canada. W90(A)

# Materials Scientist for the Oil Industry

The Esso Research Centre at Abingdon — the company's largest technical establishment in Europe — specialises in research into fuels, lubricants and additives and also provides a technical service to the refinery, marketing and transportation operations of the company.

We are shortly taking delivery of our first scanning electron microscope with microprobe analyser which will form the basis for an important new materials science facility for Esso in Europe.

We are looking for a Materials Scientist to take charge of the setting-up of this new facility and quickly integrate it with existing and future research programmes.

Aged 30-40 you should have a degree, preferably in materials science or metallurgy and at least five years' experience in the use and application of S.E.M. and other surface analytical techniques. Ideally this experience would have been in the petroleum industry or a related technical field such as Tribology.

A key attribute will be your ability to represent the potential of surface analytical techniques to customers and to establish a comprehensive service which meets Esso's future needs.

For this senior and challenging position a competitive salary will be supplemented by a full range of benefits, and generous relocation assistance, where appropriate, to this attractive rural area — just an hour away from London and only twenty minutes from Oxford.

Please write, with full details, to D.P. Sweeney, Employee Relations Service, Esso Research Centre, Abingdon, Oxon OX13 6AE, or phone him on: Abingdon 21600.



## RESEARCH

681(A)

## The University of Sussex TEMPORARY LECTURER IN HUMAN SCIENCES

One full-time, or two part-time, posts from October 1, 1979 for two years, in the School of Cultural and Community Studies. Applicants should be competent to take prime responsibility for the teaching of courses in two or more of the following areas: Human Ecology, Biosocial Anthropology, Human Prehistory and Primate Biology.

Salary will be on the Lecturer scale (full-time scale from 1.10.79: £3,975 to £8,250 per annum, under review) plus U.S.S.

Further particulars and application form, returnable by May 18, 1979, obtainable from the Establishment Section, Office of Arts and Social Studies, Arts Building, University of Sussex, BRIGHTON BN1 9QN (Brighton 606755, ext. 1050, Miss Pratt) quoting reference 034/N. 639(A)

## UNIVERSITY OF OTAGO Dunedin, New Zealand CHAIR OF PHARMACOLOGY

The University Council invites applications from medical or science graduates for appointment to the Chair of Pharmacology at present occupied by Professor F. N. Fastier who is retiring in early 1980.

Professorial salaries which are regularly reviewed, are paid within the following ranges:—

Medical NZ\$30,365 to NZ\$33,865 per annum.

Science NZ\$23,865 to NZ\$29,865 per annum.

(Both rates inclusive of NZ\$365 per annum cost-of-living allowance).

Further particulars are available from the Secretary General, Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University.

Applications close on June 30, 1979. 610(A)

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01-831-6901**

## AGRICULTURAL RESEARCH COUNCIL FOOD RESEARCH INSTITUTE

*Chemistry and Biochemistry Division*  
(Biopolymers and Physical Methods Groups)

### PROTEIN BIOCHEMIST/CHEMIST PHYSICAL CHEMIST/COLLOID SCIENTIST BIOPHYSICIST/PHYSICAL BIOCHEMIST

A **PROTEIN BIOCHEMIST OR CHEMIST** is required to initiate a programme of research into the structure and properties of food proteins—especially those of vegetable origin. The person appointed will be expected to lead a small research team and to develop techniques to study the reactions of food proteins that are important in determining the quality of food. (Ref: 78/13).

A **PHYSICAL CHEMIST OR COLLOID SCIENTIST** is required to initiate a programme of research to define the physical properties of dispersions and gels in food and to study the surface properties and interfacial characteristics of food systems. The person appointed is expected to lead a small research team and to develop modern techniques to study temperature-dependent changes and factors affecting the stability of multicomponent systems in food. (Ref: 78/8).

A **BIOPHYSICIST OR PHYSICAL BIOCHEMIST** is required to lead a small research team to study the behaviour of food biopolymers in solution or dispersion and in gels. The person appointed is expected to develop modern physical and spectroscopic techniques to study the dynamics of changes and molecular interactions in systems containing proteins and polysaccharides. A broad background in the use of physical methods in biology and familiarity with the latest developments in spectroscopic techniques is required. (Ref: 76/10).

It is intended that the persons appointed to the above posts will work in close collaboration. A broad approach is required and the ability to extrapolate from basic studies to practical problems will be an advantage.

#### Qualifications

For appointment as Higher Scientific Officer: a First or Upper Second Class Honours Degree in an appropriate subject, at least two years' relevant post-graduate experience and preferably a Ph.D. degree, or equivalent experience.

For appointment as Senior Scientific Officer: as for Higher Scientific Officer but with at least four years' relevant post-graduate experience.

Candidates with several years' postdoctoral experience and an established research record in the relevant field will be considered for appointment as Principal Scientific Officer.

#### Salary (under review)

Principal Scientific Officer £6,609—£8,461  
Senior Scientific Officer £5,154—£6,898  
Higher Scientific Officer £4,101—£5,448

(Starting salary according to experience).

Non-contributory superannuation scheme at age 18 years and over.

Five-day week and flexible working hours scheme operated.

**Application form and further particulars from the Secretary, Food Research Institute, Colney Lane, Norwich NR4 7UA quoting appropriate reference. Closing date: May 21, 1979.**

628(A)

## Liverpool Area Health Authority (T)

Regional Cardiac Centre, Sefton General Hospital,  
Smithdown Road, Liverpool L15 2HE

## BIOCHEMIST (Basic Grade)

Applications are invited for the post of Biochemist (Basic Grade) in the Centre Laboratory, which is concerned with biochemical aspects of heart disease.

Whitley Council conditions of pay and service apply. Newly qualified graduates will be considered.

**Application forms obtainable from the Sector Administrator, Sefton General Hospital, Smithdown Road, Liverpool L15 2HE. Closing Date: 15th June 1979.**

590(A)

## CONFERENCES

### CALL FOR ABSTRACTS BIOPHYSICAL DISCUSSIONS Second Discussion—May 1980 PROTEINS AND NUCLEOPROTEINS: STRUCTURE, DYNAMICS AND ASSEMBLY

The Biophysical Society will hold its 2nd Biophysical Discussion at Airlie House, Airlie, Virginia (near Washington, D.C.) on May 18-21, 1980. This Discussion will consider recent advances in understanding the principles of macromolecular structure and dynamics. Sessions will be devoted to: elucidation of structure by diffraction and spectroscopic methods; nature of forces stabilizing macromolecular structure; fluctuations in macromolecular structures; and mechanisms of folding and assembly. Experimental systems to be discussed include proteins, viruses, and organelles involving protein-nucleic acid interactions.

Prior to the meeting, all participants will receive a study book containing the full Discussion papers and poster abstracts. There will be no formal presentation of papers at the meeting, only a five-minute reminder followed by open discussion. A \$175 fee will cover registration, three days' room and board, the study book, and the final proceedings volume.

Papers submitted for the Organizing Committee's consideration are due as follows:

July 16, 1979—Preliminary abstracts (<300 words, to be reviewed and selected by August 1st)  
December 1, 1979—Complete manuscripts (to be refereed and selected by mid-January)

The full edited proceedings of ca. 500 pp, will be published in hardback by Rockefeller University Press (\$20 prepublication, \$30 after October 1980). Identical text will be received by *Biophysical Journal* subscribers (1980 subscription, 12 issues, \$150). For these publications, remit to Order Service, Rockefeller University Press, P.O. Box 5108, New York, N.Y. 10249, USA.

For further information contact Valerie Parsegian, Executive Secretary, Biophysical Discussions, P.O. Box 30239, Bethesda, Maryland 20014, USA. Phone (202) 362-8184. W93(C)

Quekett Microscopical Club Lecture. Dr James Dyson, Sc.D., F.R.S., will speak on 'Interference and Image Shearing Microscopy' at 6.30 p.m. on Tuesday, May 8, at the British Museum (Natural History), Cromwell Road, South Kensington, London SW7 5BD. All Club members and visitors are welcome. 563(C)

## STUDENTSHIPS

### UNIVERSITY OF OXFORD M.R.C. RESEARCH STUDENTSHIP

Applications are invited from biochemists, chemists or physiologists for a 3 year studentship to commence in October 1979. The successful candidate will register for a D. Phil and pursue research into the chemistry, kinetics and mechanism of enzymes involved in human cataract formation, in particular aldose reductase in diabetes.

Applications together with the names and addresses of three referees, and requests for further information should be sent to Dr James Crabbe,

University of Oxford,  
Nuffield Laboratory of  
Ophthalmology,  
Walton Street,  
Oxford OX2 6AW. 621(F)

### UNIVERSITY OF EAST ANGLIA BP RESEARCH CENTRE S.R.C. C.A.S.E. STUDENTSHIP

Infrared Spectroscopy of Reaction Intermediates on Catalyst Surfaces in Propylene Ammoxidation. S.R.C. Research Studentship plus £100 plus fees available from October 1, 1979.

Apply to Professor N. Sheppard, F.R.S., School of Chemical Sciences, University of East Anglia, Norwich NR4 7TJ. 648(F)

## UNIVERSITY OF CAMBRIDGE

### DEPARTMENTS OF APPLIED BIOLOGY AND MINERALOGY N.E.R.C./C.A.S.E. RESEARCH STUDENTSHIP

Applications invited from scientists holding or expecting to obtain a good honours degree for a project on the identification, origin and reaction of asbestos waste in ground-water systems, starting October 1979.

Further details from Dr. C. V. Jeans, Department of Applied Biology, Pembroke Street, Cambridge CB2 3DX. 624(F)

### THE MIDDLESEX HOSPITAL MEDICAL SCHOOL (University of London) MEDICAL RESEARCH COUNCIL STUDENTSHIP

Applications are invited from a candidate with a good honours degree to work on biophysical aspects of cell-substratum interactions or membrane fusion. Applications, including curriculum vitae, and the names of two referees, to Dr David Gingell, Department of Biology as Applied to Medicine, The Middlesex Hospital Medical School, London W1P 6DB. 647(F)

### UNIVERSITY OF EAST ANGLIA Norwich S.R.C. C.A.S.E. STUDENTSHIPS

S.R.C. CASE awards will be tenable at the University of East Anglia from October 1, 1979 in support of research in the following fields:—

- a) in cooperation with the 'Glasshouse Crops Research Institute' 1. "The role of spiders in checking aphid outbreaks in cereals" Supervisors: Professor A. F. G. Dixon/Dr. K. D. Sunderland.
2. "Aphid-specific natural enemies of cereal aphids" Supervisors: Professor A. F. G. Dixon/Mr. R. J. Chambers.
- b) in cooperation with the Forestry Commission "Control of polymorphism in green spruce aphid" Supervisors: Professor A. F. G. Dixon/Dr. C. I. Carter.
- c) in cooperation with the Food Research Institute, Norwich "Infection of carrot roots by *Sclerotinia sclerotiorum*" Supervisors: Dr. B. G. Lewis/Dr. C. Dennis.

Applicants should possess or expect to obtain a First or Upper Second Class degree and should write to Professor A. F. G. Dixon, Dean School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ. Applications together with a curriculum vitae and the names of two referees should arrive by May 18 1979. 657(F)

### M.R.C. PNEUMOCONIOSIS UNIT

Llandough Hospital  
PENARTH,  
SOUTH GLAMORGAN  
CF6 1XW

Applications are invited for a MRC Research Studentship from those who have, or expect to obtain this year a good honours degree, M.Sc. or equivalent qualification in physics, electrical engineering, mathematics, or statistics. The successful candidate will register for a Ph.D.

Projects include instrument development in respiratory physiology and the study of the statistical aspects of dose response curves for hazardous substances.

Further information and application forms may be obtained from the Assistant Administrative Officer. 619(F)

## STUDENTSHIPS—continued

DEPARTMENT OF PHYSIOLOGY  
UNIVERSITY OF  
LIVERPOOL  
M.R.C.  
RESEARCH STUDENTSHIP

Applications are invited for an M.R.C. Research Studentship in the Department of Physiology. Candidates should have, or expect to obtain this year, a good honours degree in Physiology or a related subject. Experience in some aspect of histology and cell biology would be an advantage. The successful applicant will join a group working on a development and application of immunocytochemical methods to study the cellular origins, chemical relationships and physiological roles of gut hormones and neuropeptides. He/she will be expected to register for a Ph.D.

Applications, together with the names of two referees, should be received not later than June 7, 1979, by the Registrar, The University, P. O. Box 147, Liverpool, L69 3BX. Quote Ref. RV/583/N 674(F)

UNIVERSITY OF LEICESTER  
DEPARTMENT OF ZOOLOGY  
SCHOOL OF  
BIOLOGICAL SCIENCES  
POSTGRADUATE

## RESEARCH STUDENTSHIP

Applications are invited for a postgraduate research studentship financed by the Medical Research Council, and tenable for three years for the study of regulation and determination of spatial patterns of cellular differentiation in arthropods.

The study would be of particular interest to those with some interest and background in Developmental, Cell or Neurobiology, although candidates with other qualifications will be considered.

Applicants should send a curriculum vitae and names and addresses of 2 referees to Dr P. M. J. Shelton, Department of Zoology, University of Leicester, Leicester LE1 7RH, as soon as possible. 514(F)

UNIVERSITY OF  
EDINBURGH  
M.Sc. COURSE  
on The Physics and Technology  
or Amorphous Materials

(a joint course with the Universities of Dundee and Glasgow) 1 year full-time from October 8, 1978

Main topics include: Structure; Preparation; Characterisation; Basic Theory; Electronic and Optical Properties; Thermal and Mechanical Properties; Magnetism; Glasses and the Vitreous State; Technological Applications, including Solar Energy Conversion and Applications in Solid State Electronics. The final term will be devoted to an individual research project.

Entry qualifications: Normally an honours degree or its equivalent in Physics, Electrical Engineering, Chemistry or a Materials Science.

Grants: SRC Advanced Course Studentships are available for applicants with appropriate qualifications. Further information from: Dr A. E. Owen, Department of Electrical Engineering, King's Buildings, University of Edinburgh, Edinburgh, EH9 3JL, Scotland, U.K. 673(D)

UNIVERSITY OF GLASGOW  
DEPARTMENT OF ORGANIC  
CHEMISTRY  
POSTDOCTORAL  
FELLOWSHIP IN  
BIOORGANIC CHEMISTRY

Available for two years from October 1, 1979 to study amino-acid metabolism in plant tissue cultures and micro-organisms. SRC-sponsored. Applicants should have strong background in organic synthesis. Experience with radio-isotopes and micro-organisms or plants an advantage but not essential. Starting salary in range £3,883 to £4,382 per annum (1A, Research and Analogous Staff salary scales—currently under review). Applications with names of two referees to Professor K. H. Overton, Chemistry Department, University of Glasgow, Glasgow, G12 8QQ. In reply please quote Ref. Number 4436M. 596(E)

INSTITUTE OF  
CANCER RESEARCH  
(Chester Beatty Research  
Institute)

MASS SPECTROMETRY—  
DRUG METABOLISM GROUP

A three-year postdoctoral fellowship will be available later in 1979 associated with a multidisciplinary group studying the metabolism of anticancer drugs in relation to mode of action, design of new drugs, and improvement of clinical use. In vitro, animal, and human studies are in progress. A major current emphasis concerns the study of a group of drugs which are in current clinical use for the treatment of disseminated breast cancer. The appointee will be responsible for that part of the Group programme which involves the use of desmolase (cholesterol side chain degradation) aromatase, and estrogen receptors in developing structure-activity relationships. Applicants should have research training in biochemistry, preferably with an interest and experience in enzyme manipulation, and not more than two years' postdoctoral experience. The fellowship, which is superannuated (U.S.S.), will have an initial salary (presently under review) of not less than £4,910 per annum (including London Allowance).

Applications, including a full curriculum vitae, in duplicate and with the names of two referees should be sent as soon as possible to the Secretary, Institute of Cancer Research, 34 Sumner Place, London SW7 3NU, quoting ref. 300/G/5. 649(E)

## COURSES

UNIVERSITY OF SOUTHAMPTON  
Department of Chemistry

## M.Sc. Course in Electrochemical Science

Applications from candidates with a good honours degree are invited for this 12 month course, which provides a comprehensive training in the methodology of modern electrochemical research and the applications of electrochemistry in industry. The course, which commences in October 1979, has been approved by the Science Research Council, and a number of studentships will be available. Further details of the course and application forms can be obtained from Dr L. M. Peter, Department of Chemistry, The University, Southampton, SO9 5NH/N. 656(D)

## FELLOWSHIPS

QUEEN'S UNIVERSITY  
Kingston, Ontario

Applications are invited for a M.R.C. supported postdoctoral fellowship to work with Dr B. T. Eaton on the study of arbovirus persistence in mosquito cells. The appointment is available for two years and the initial salary is \$12,000 per annum. Prospective or recent Ph.D. graduates should send their curriculum vitae and the names of two referees to Dr Eaton, Department of Microbiology and Immunology, Queen's University, Kingston, Ontario K7L 3N6, Canada. W80(E)

NATIONAL INSTITUTE FOR  
MEDICAL RESEARCH  
POSTDOCTORAL  
RESEARCH FELLOWSHIP

Applications are invited from suitably qualified biochemists, pharmacologists or neurochemists to join a team who are investigating the biochemical and pharmacological properties of muscarinic acetylcholine receptors in the Division of Molecular Pharmacology. This position is available immediately and will be renewable on an annual basis for at least two years. The salary will be the same as that of a member of the Medical Research Council scientific staff of comparable age and experience.

Applicants should send curriculum vitae and the names of two referees to Dr N. J. M. Birdsall, Division of Molecular Pharmacology, National Institute for Medical Research, Mill Hill, London NW7 1AA. 620(E)

THE UNIVERSITY OF  
MANCHESTER  
POSTDOCTORAL  
RESEARCH FELLOWSHIP IN  
OFFSHORE ENGINEERING

Applications are invited for research into the behaviour of offshore piles subjected to cyclic loading using the 700g tonne centrifuge. The appointment is for 2½ years commencing September 1, 1979 at a salary of up to £6,080 per annum (under review). Superannuation. Full particulars of the post are obtainable from Mrs. A. C. Davies, Simon Engineering Laboratories, The University, Brunswick Street, Manchester. 655(E)

UNIVERSITY OF SUSSEX  
SCHOOL OF  
MOLECULAR SCIENCES  
POSTDOCTORAL  
RESEARCH FELLOWSHIP

Applications are invited for an S.R.C. Research Fellowship in the School of Molecular Sciences to work on Cycloadditions to Silyl-protected Polyacetylenes and Polyenes in collaboration with Dr D. R. M. Walton. The position will become available on October 1, 1979 and is within the usual Research Fellow 1A salary range. The appointment is for one year in the first instance, renewable for a further year. Small-scale preparative research experience in organometallic/natural product chemistry is desirable, coupled with a genuine interest in p.m.o. calculations.

Applications, accompanied by the names of two referees should be sent as soon as possible to Mr R. W. Bott, Sub-dean, School of Molecular Sciences, University of Sussex, BN1 9QJ. 638(E)

M.Sc. BRAIN STUDIES  
BRUNEL UNIVERSITY

Two-year part-time course in Cybernetics, Experimental Psychology and Neurophysiology. Entries October 1979 and October 1981.

Applicants should have 1st or 2nd class honours degree in a biological, medical or mathematical subject. Details from Dr R. C. Elliott, School of Biological Sciences, Brunel University, Uxbridge, Middlesex. 609(D)

MODERN TECHNIQUES  
IN CENTRIFUGATION  
September 2-7, 1979  
UNIVERSITY OF ESSEX

This is a workshop course designed to give participants practical experience of the newer centrifugation techniques now available. Participants will be able to use a wide variety of centrifuges and rotors including vertical rotors. The practical sessions will be complemented by a series of lectures and discussion groups covering topics of current interest. For further information contact the Liaison Officer (N), University of Essex, Wivenhoe Park, Colchester, CO4 3SQ (Tel. Colchester 862286, ext. 2375) 623(D)

BIRKBECK COLLEGE  
(University of London)

M.Sc.  
NATURAL ENVIRONMENTS  
AND PLANT GROWTH  
An integrated part-time EVENING  
COURSE in Soil Hydrology,  
Microclimatology and Plant  
Ecology

The M.Sc. Natural Environments and Plant Growth consists of a course of lectures, laboratory practicals and field-work extending over two academic years of part-time study and is given within the departments of Geography and Botany. Three modules are offered: Boundary Layer and Plant Canopy Microclimatic Environment; Soil Hydrological Environment; Environment and the Growth and Development of Plants.

Application forms and further details may be obtained from: The Secretary, Department of Geography, Birkbeck College, 7-15 Gresse Street, London W1P 1PA. 662(D)



## FELLOWSHIPS—continued

★ UNIVERSITY OF GLASGOW  
DEPARTMENT OF  
CHEMISTRY  
POSTDOCTORAL  
FELLOWSHIP

The above position is available under an M.R.C. project grant in respect of the determination of the structure of a bacterial chloramphenicol acetyl transferase at atomic resolution. This study is well advanced and the post is open to applicants with experience in crystallography. Starting salary will be within Range 1A of the salary scale for Research and Analogous Staff, £3,883 to £4,382, depending on age and experience.

Further particulars are available from Dr I. D. A. Swan, Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, to whom applications, together with a curriculum vitae and the names of two referees, should be addressed.

In reply please quote Ref. No. 4325/1. 629(E)

## ASSISTANTSHIPS

CITY OF LONDON  
POLYTECHNIC  
DEPARTMENT OF BIOLOGICAL  
SCIENCES  
RESEARCH  
ASSISTANTSHIPS (2)

Applications are invited for two Research Assistants to work on the following projects:

1. To study bacteria from anaerobic environments, their interactions and effects on metal corrosion and the influence of inhibitors. The project will include adsorption studies, involving the use of the scanning electron microscope and radioactive tracer techniques. Reference number 79/29.
2. The regulation of gene expression during muscle differentiation with particular emphasis on a study of muscle nuclei. Applicants should have a background in biochemistry or Cell Biology. Reference number 79/28.

The appointments will be tenable for two years in the first instance and will be extended to three on satisfactory completion of the first two years.

The salary scale for Research Assistants is £2,613 to £2,700 to £2,787 plus London Allowance of £474 per annum.

Please apply in writing, giving full curriculum vitae and the names and addresses of two referees to the Staff Records Officer, City of London Polytechnic, 117 Houndsditch, London EC3A 7BU. Please quote the appropriate reference number.

663(P)

UNIVERSITY OF ABERDEEN  
DEPARTMENT OF ZOOLOGY  
M.R.C. RESEARCH  
ASSISTANTSHIP

Applications are invited for a Research Assistant to examine the relationship between surface-bound antibody and nutrient absorption in tapeworms. Candidates should have an Honours degree in either Immunology or Biochemistry (with experience in immunology techniques).

The appointment is tenable until April 30, 1981 from an early date, to be arranged.

Salary within Range 1B, £3,384 to £3,883 per annum (under review), with appropriate placing.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by May 25, 1979. 622(P)

UNIVERSITY OF  
CAMBRIDGE

DEPARTMENT OF BOTANY  
RESEARCH IN GENETICS

Applications are invited for a postdoctoral research assistantship to study aspects of genetic recombination using fungal spore colour mutants. The appointment is supported by the S.R.C. and is for two years. Starting salary not less than £3,883 per annum. Apply to Dr. H. L. K. Whitehouse, Botany School, Downing Street, Cambridge CB2 3EA, giving the names of two referees. 653(P)

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572(L)

## MISCELLANEOUS

Dr Philippe KOURILSKY of the Unité de Génie Génétique, Institut Pasteur, Paris, will address the Annual Meeting of the Louis Rapkine Association on 'Génie Génétique—Problèmes et Perspectives' Friday, May 11, 1979 at 6 p.m. at the French Institute, Queensberry Place, London SW7. Admission free. 625(J)

## SYMPOSIA

SYMPOSIUM ON  
REPRODUCTIVE LOSS

The tenth annual New York State Health Department Birth Defects Symposium will be held in Albany, New York on October 29 to 31, 1979 in the Rockefeller Empire State Plaza Convention Center.

Address Inquiries to:  
Ian H. Porter, M.D.,  
Director, Birth Defects Institute,  
New York State Health Dept.  
Room 1917,  
Tower Building,  
Rockefeller Empire State Plaza,  
Albany, New York 12237.

W88(N)

FIFTH EMBO  
ANNUAL SYMPOSIUM

NUCLEIC  
ACID-PROTEIN  
INTERACTIONS

October 12–15 inclusive  
EMBL Heidelberg

In addition to members of the Organising Committee invited speakers include: J. Abelson (La Jolla); T. Bickle (Basel); H. Bujard (Heidelberg); P. Chambon (Strasbourg); R. Flavell (Amsterdam); C. Guthrie (San Francisco); S. Harrison (Harvard); C. Helene (Orléans); H. Hoffmann-Berling (Heidelberg); A. Landy (Brown); A. McPherson (Pennsylvania); M. Ptashne (Harvard); M. T. Record (Madison); H. Saedler (Freiburg); A. Schmitz (Geneva); J. Sperling (Rehovot); G. Stöffler (Berlin); F. W. Studier (Brookhaven); E. Vinuela (Madrid); J. C. Wang (Harvard); W. Wintermeyer (Munich); C. Yanofsky (Stanford).

The eight Plenary Sessions will cover Model Systems; Viruses; Chromatin; Polymerases; Regulatory Signals on DNA; Cleaving, Processing and Modifying Nucleic Acids; Ribosomes and tRNA; New Frontiers.

The Symposium will be held at the European Molecular Biology Laboratory Heidelberg with registration on Thursday October 11. Plenary sessions will be held in the mornings and afternoons of October 12-15 inclusive. The Symposium banquet will be held on the evening of October 15. Poster sessions will be arranged; applicants wishing to present a poster should submit a title and an abstract of 1-2 pages. Participants will be accommodated in two hotels in downtown Heidelberg, and transportation to the meeting will be arranged.

The registration fee is DM 80,— and includes daily lunches and the banquet but neither hotel charges nor evening meals.

Applications including a curriculum vitae should be sent to Dr. J. Tooze, Executive Secretary, European Molecular Biology Organisation, Postfach 1022.40, 69 Heidelberg 1, FRG, by May 31, 1979. Since the number of participants is limited to 200 the Organising Committee will notify those who have been accepted in August.

Organising Committee: D. Crothers (Yale); J.-P. Ebel (Strasbourg); A. Klug (Cambridge); C. Kurland (Uppsala, Chairman); J. Miller (Geneva); H. Schaller (Heidelberg); J. Tooze (Heidelberg, Secretary); C. Weissmann (Zürich).

W86(M)

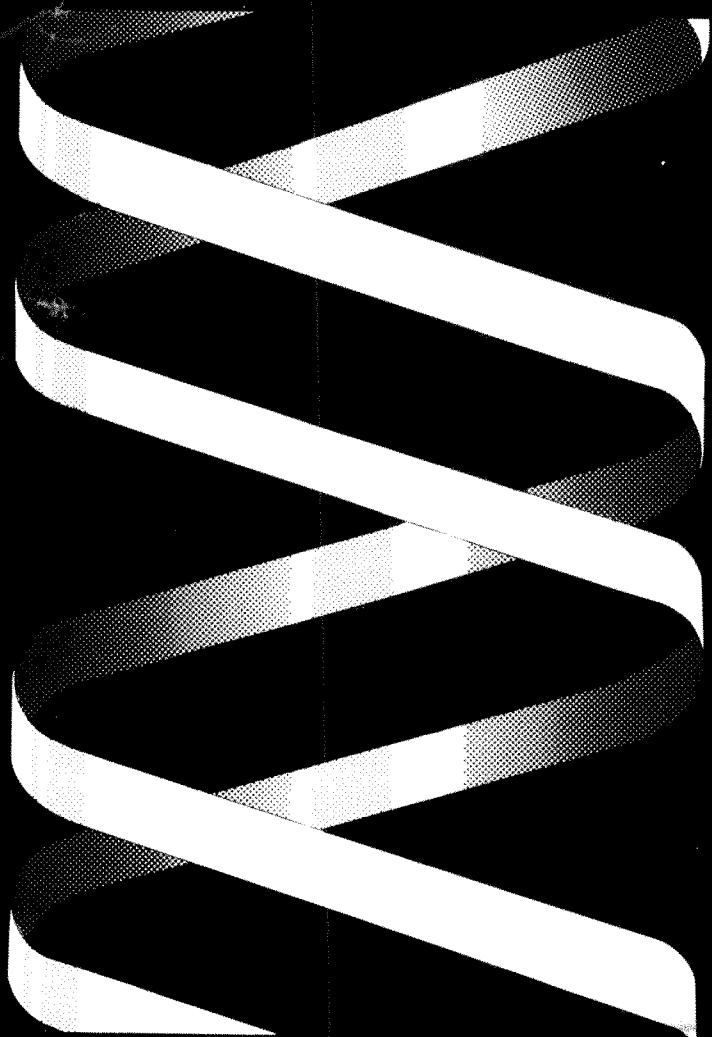
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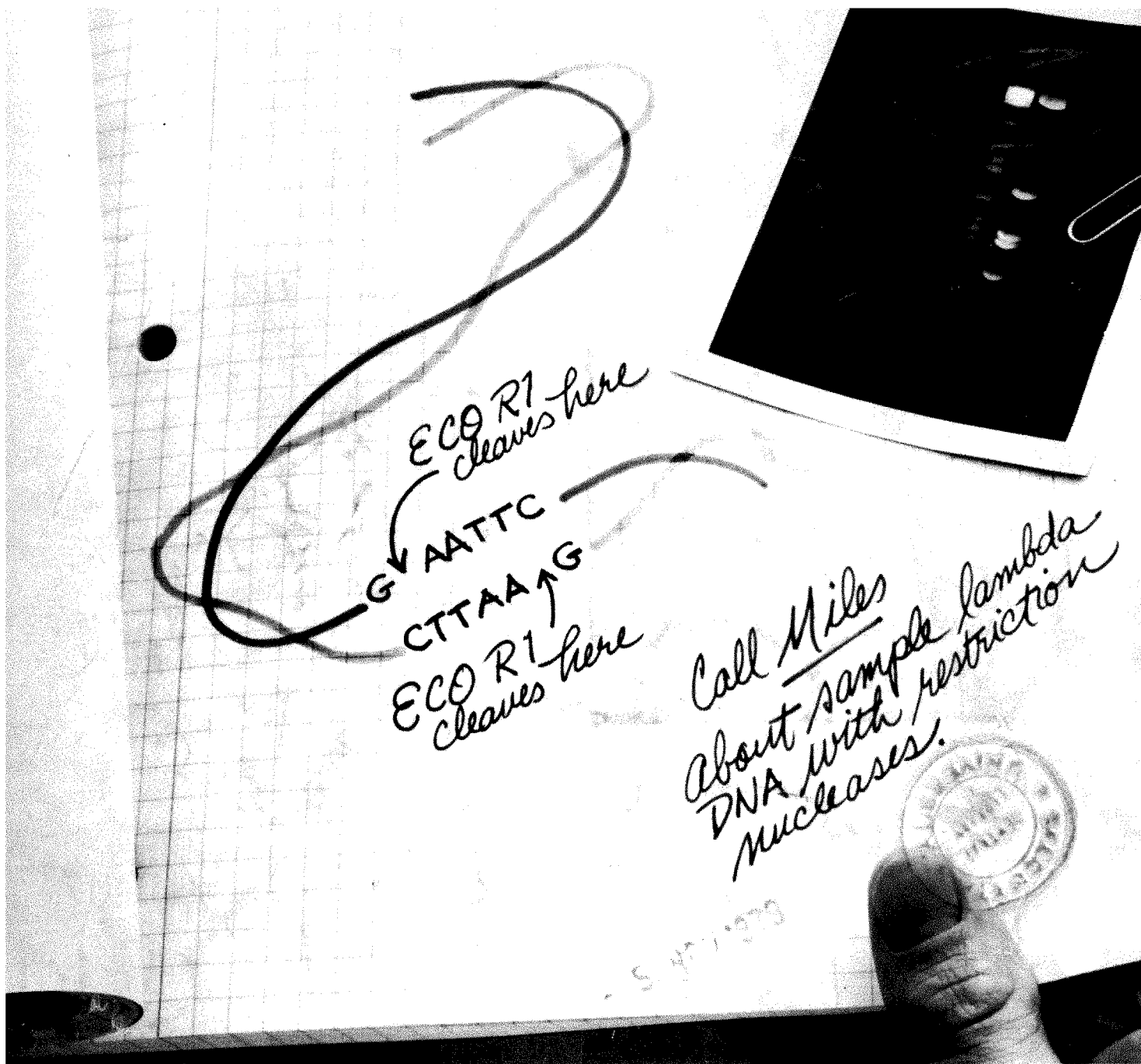
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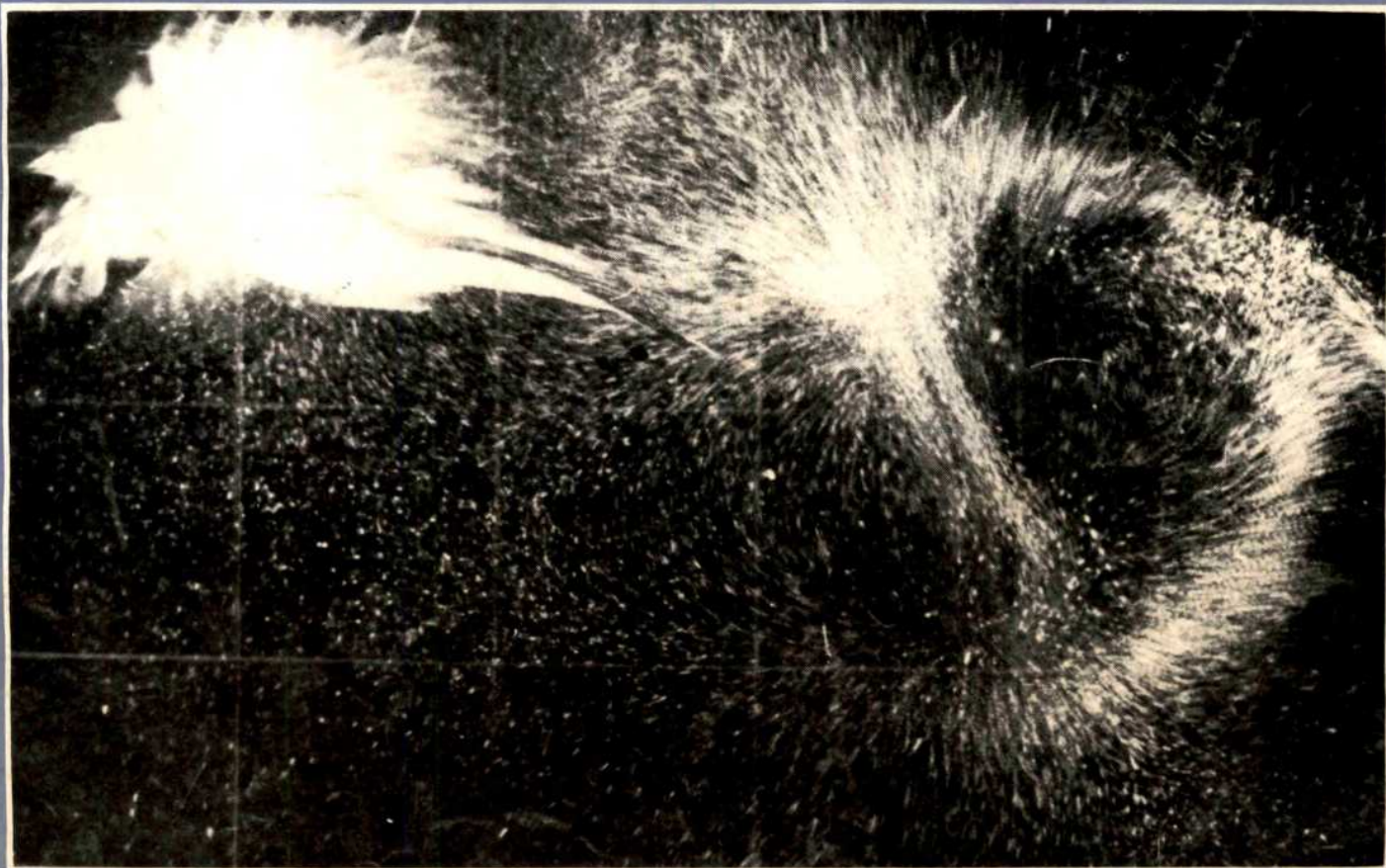
# nature

Vol 279 No 5709 10 May 1979 80p US \$2.25

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*The wake of  
a flying bird*





# Boehringer Mannheim the enzyme people present

ate de- se- late uta- lyo- oxi- leu- P1, ate nate Dro- lase, pha- calf beef en), om), hos- mate iso- ase, holi- oli- ase nos- me- seb, otide lase, Pro- tease se K, pep- anic, onu- ease Eco id II, III, II, stic- trans- bonu- bonu- orbitol cynyl- rase, iose- scl), east), ricase Jridi- enase, phos-	Bam HI, Restriction endonuclease Hind II, R I, Restriction endonuclease Hind III, Restriction endonuclease Hpa II, Acetate kinase, Acetylcholinesterase, Acetyl-CoA synthetase, N-Acetyl-β-D-glucosaminidase, Adenosine deaminase, L-Alanine dehydrogenase, Alcohol dehydrogenase (horse liver), Alcohol dehydrogenase (yeast), Aldehyde dehydrogenase, Aminoacylase, Amino Aldolase, D-Amino acid oxo acid arylamidase, α-Amylase, L-Amino acid oxidase, α-Amylase (Bacillus subtilis), α-Amylase (porcine pancreas), β-Amylase, Amyloglucosidase, L-Arginase, Arylsulphatase, Ascorbate oxidase, L-Asparaginase, Bromelain, Carbonic anhydrase, Carboxypeptidase A, Carboxypeptidase B, Carboxypeptidase Y, Carnitine acetyltransferase, Catalase, Cathepsin C, Cellulase, Cholesterol esterase, Cholesterol oxidase, Chymotrypsin A, Citrate lyase, Citrate synthase, Clostridinase, Collagenase, Creatine kinase, Creatininase, Cytochrome c, Deoxyribonuclease I, Diaphorase, Disphase bonuclease (neutral), DNA-polymerase (Kornberg-polymerase), DNA-polymerase (enzyme A according to Klenow), Elastase, Elongation factor Tu-Ts, Endonuclease, Enolase, Esterase, Ficin, Formate dehydrogenase, Fructose-6-phosphate kinase, α-L-phosphate kinase, β-Fructosidase, α-L-phosphatase, Fumarase, β-Galactose dehydrogenase, α-Galactosidase, dehydrogenase, Gluconate kinase, β-Galactosidase, Glucose-6-phosphate dehydrogenase (yeast), Glucose-6-phosphate dehydrogenase (Leuconostoc mesenteroides), α-Glucosidase, β-Glucosidase, β-Glucuronidase, β-Glucuronidase, Glutamate dehydrogenase, Glutamate-oxaloacetate transaminase, Glutamate-pyruvate transaminase, Glutathione reductase, Glyceraldehyde-3-phosphate dehydrogenase (rabbit muscle), Glyceraldehyde-3-phosphate dehydrogenase (yeast), dehydrogenase (hog muscle), Lactate dehydrogenase (pig heart),	Protease (neutral, B. polymyxa), Protease (nonspecific, St. griseus), Protease (submaxillaris glands), Proteinase K, Pullulanase, Pyroglutamate aminopeptidase, Pyrophosphatase, inorganic, Glycerokinase, Glycerol dehydrogenase, Glycerol-3-phosphate dehydrogenase, Glyoxalase I, Glyoxylate reductase, Guanase, Guanosine-5'-monophosphate kinase, Hexokinase, Hyaluronidase, 3-Hydroxyacyl-CoA-dehydrogenase, 3-Hydroxybutyrate dehydrogenase, 20β-Hydroxysteroid dehydrogenase, Isocitrate dehydrogenase, D(-)-Lactate dehydrogenase, Lactate dehydrogenase (beef heart), Lactate dehydrogenase (beef muscle), Lactate dehydrogenase (hog muscle), Lactate dehydrogenase (pig heart), Lactate dehydrogenase (rabbit muscle), Lactoperhydrogenase (rabbit muscle), Lactoperoxidase, Leucine aminopeptidase, Lipase, Luciferase, Lysozyme, Malate dehydrogenase, α-Mannosidase, Mutarotase, Myokinase (hog muscle), Myokinase (rabbit muscle), NADH-peroxidase, NAD-pyrophosphorylase, Neuraminidase, Nuclease, Nuclease P1, Nuclease S1, Nucleoside-5'-diphosphate kinase, Nucleoside monophosphate kinase, Nucleoside phosphorylase, Orotidine-5'-phosphate pyrophosphorylase, Papain, Pepsin, Peroxidase, Phosphatase (acid), Phosphatase (alkaline, calf intestine), Phosphodiesterase (beef heart), Phosphodiesterase (calf spleen), Phosphodiesterase (snake venom), Phosphoenolpyruvate carboxylase, Phosphoglucosylase, 6-Phosphogluconate dehydrogenase, Phosphoglucose isomerase, 3-Phosphoglycerate kinase,	Phosphoglycerate mutase, Phospholipase A <sub>2</sub> (porcine pancreas), Phospholipase A <sub>2</sub> (snake venom), Phospholipase A <sub>2</sub> (bee venom), Phospholipase C, Phospholipase D, Phosphomannose isomerase, Phosphorylase a, Phosphorylase b, Phosphotransacetylase, Polynucleotide kinase, Polynucleotide phosphorylase, Protease (neutral, B. polymyxa), Protease (nonspecific, St. griseus), Protease (submaxillaris glands), Proteinase K, Pullulanase, Pyroglutamate aminopeptidase, Pyrophosphatase, inorganic, Pyruvate kinase, Restriction endonuclease Alu I, Restriction endonuclease Eco Bam HI, Restriction endonuclease Hind II, R I, Restriction endonuclease Hind III, Restriction endonuclease Hpa II, Restriction endonuclease Pst I, Restriction endonuclease Sma I, Reverse transcription endonuclease, Ribonuclease, Ribonuclease A, Ribonuclease T <sub>1</sub> , Ribonuclease U <sub>2</sub> , RNA-polymerase, Sorbitol dehydrogenase, Succinyl-CoA synthetase, Terminal transferase, Thermolysin, Transaldolase, Triose-phosphate isomerase (rabbit muscle), Triosephosphate isomerase (yeast), Trypsin, Urease S, Urease, Uricase (hog liver), Uricase (microbial), Uridine-5'-diphospho-glucose dehydrogenase, Uridine-5'-diphospho-glucosepyrophosphorylase, Uridyltransferase, Xanthine oxidase,	Acetate kinase, Acetylcholinesterase, Acetyl-CoA synthetase, N-Acetyl-β-D-glucosaminidase, Adenosine deaminase, 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### Cover picture

The wake of a bird visualised by taking multiframe photographs of a bird flying through a cloud of wood-or paper-dust. See page 146 for a study of the general configuration of flapping bird flight.

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nature

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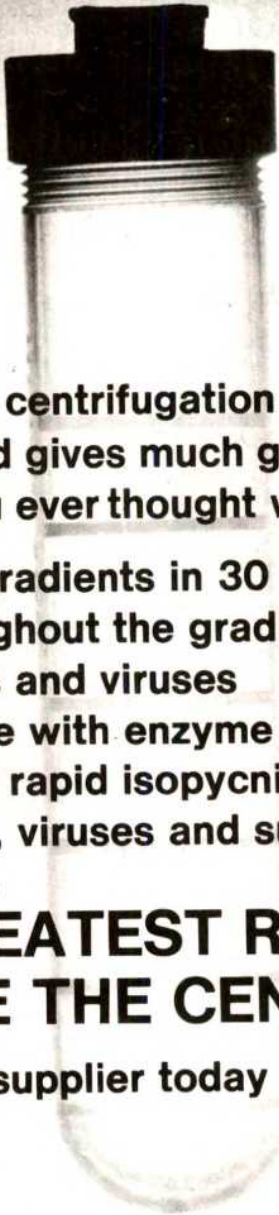
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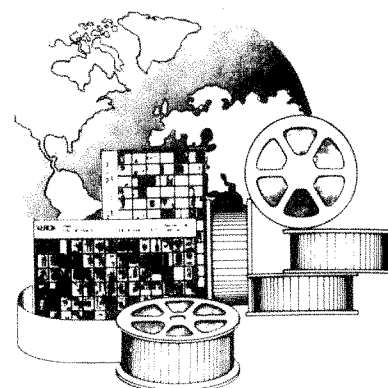
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nature

10 May 1979

## Wasted talent is wasted opportunity

THE Conservative and Unionist Party has been elected to govern the United Kingdom, with Mrs Thatcher as Prime Minister. The election was fought almost entirely on economic issues, and even on these the new government communicated little more than an intention. (We heard nothing about how much income tax would be cut, or what rise in value added tax would be necessary to pay for it, except for Labour's own, presumably biased, calculations.) Of science we have heard nothing—except on environmental issues from the Ecology Party, and on nuclear power from the Liberals.

We hope that the new government will recognise the central importance of science—and basic science, at that—to the economy. In opposition, the Conservatives have frequently called attention to 'wealth creation', which it says Labour has ignored. The Conservatives appear prepared to increase social inequalities (by redistributing the tax burden) and risk confrontation with organised labour (by introducing new legislation and cutting 'lame duck' industrial support grants). But are they prepared to invest in science? It is undeniable that science is increasingly the true source of wealth, now that the days of empire and exploitation are receding. President Carter has recognised this, with his constant calls to Congress for an increase in science funding. And West Germany has recognised it, with a massive increase in funding for its science ministry in this year's budget.

Shirley Williams, the outgoing Secretary of State for Education and Science, had also recognised it, managing to wrest a few extra millions for the research councils this year. But will the Tories recognise it? We hope they will have the vision to do so, particularly with Britain's first scientist (Mrs Thatcher was a chemist) as Prime Minister. Mrs Williams' balm merely alleviated the worst of a long history of financial stringency in the research councils, and there is a great deal of slack to be taken up—not least in the replacement of aging equipment like spectrometers and microscopes, or proper investment in Britain's great scientific successes like molecular biology (where with genetic engineering there is enormous economic potential) or even the less obviously economic radio and high energy astronomy. Great international centres of excellence have a galvanising effect on the science of a country that goes far beyond their particular discipline. Even a geophysicist, for example, feels pride that the double helix was discovered in this country.

There is no doubt that science in the UK is depressed. This is the time to revitalise it. The argument that basic science should be left for better times is false. The country that now invests deeply in science, despite the state of the economy, will be the one that emerges from the present world recession ready to take the lead.

Furthermore, it's not just a question of money. If wealth creation is a matter of science (and its application through

engineering), science is a matter of scientists. And Britain has never had a coherent policy for the growth and encouragement of its scientific community—just the usual British compromise of a decision here, a regulation there, a patchwork but no policy. The research councils have done their best to regulate and redirect studentships and fellowships; but they can only tinker with the problem. There is an enormous—perhaps 5,000-strong—and vital community of bright researchers locked into a cycle of frustrating and demoralising short-term contracts, produced by the unwritten policy of using an excessive number of research students as cheap labour and the static university system blocked with middle-young tenured staff. Of course 'the best' always get jobs—that's only a matter of circular definition. The real question is of the wasted talent, the real talent, that does not find effective employment, either in the universities or in industry.

This problem is not Britain's alone. Even Germany suffers from it, despite its enlightened attitude to science. But the country that breaks through the logjam first will have an immense advantage. We challenge the new government to find effective, creative employment for Britain's post-doctoral fellows. A dozen new whizz-kid centres of advanced study, some applied, some pure? A proper career structure for non-teaching researchers at universities and medical schools would be a good start. The objective must be to create new research schools, either attached to universities as non-teaching institutes or completely independent. Research doesn't grow at random. It depends on potentially creative branches having the opportunity to develop at the right moment. The United States took its great lead in science when Europe rejected its intellectuals during the time of Hitler; they landed in America and research communities spread rapidly around them.

US pre-eminence in theoretical sub-nuclear physics, for example, can be traced largely back to Enrico Fermi's school in Chicago. And how did Britain invent its way through the second world war? It gave men like R. V. Jones responsibility the moment they could shoulder it.

We should take exactly the same attitude to our post-doctoral community. It must be given the opportunity to spawn its own schools of research, in an atmosphere where there is—if not total job security—at least hope, and growth, and vision. The age of this community—reaching into the mid thirties—is just right to bear fruit. (Studies by Harriet Zuckerman, recorded in her book *Scientific Elites* (Free Press, New York: 1977), show that the average age at which Nobel prize-winners did their prize-winning work was around 36. Those few who were under 30 were usually already members of flourishing research schools.)

So we say this to our new Prime Minister—whatever your successes or failures with industry and the more immediate problems of the nation: remember the scientists—and you will be remembered. □

# US academy denies threshold for radiation damage

A COMMITTEE of the US National Academy of Sciences, after many months of heated debate, last week confirmed its support for the hypothesis that ionising radiation has an effect on the human body that remains directly proportional to the dose even at very low levels, and that there is therefore no threshold below which such radiation can be ignored.

This decision has been reached by the academy's Committee in the Biological Effects of Ionising Radiation—(BEIR) which had been asked by the Environmental Protection Agency to reconsider estimates of the hazards of ionising radiation first made in a report published by the committee in 1972.

Pointing out the absence of clear scientific evidence on the shape of the dose-response curve for low levels of radiation—a source of much recent controversy over, for example, the hazards faced by workers in nuclear shipyards and power stations—the committee says that the linear dose-response relationship “emerges by default as the simple model whose use appears to be the least objectionable”.

This conclusion, however, and the estimates of increased cancer incidence to which it leads, is sharply contested by a number of committee members, who state in a minority report signed by five of the 22 members that “far too little theoretical information exists to serve as a reliable guide for extrapolation.”

Speaking at a press conference held by the NAS to present the report, Dr Harald H. Rossi of the Columbia University College of Physicians and Surgeons in New York, one of the minority group, said he feared that the report would “contribute to excessive, and potentially detrimental, apprehension over radiation hazards.”

In reply, Dr Edward Radford, of the University of Pittsburgh's Graduate School of Public Health, chairman both of the BEIR committee and of its somatic effects subcommittee, issued a public challenge to any member of the minority group, or any other individual, “to meet me in public debate to discuss these issues openly and to see who comes nearest to the truth.”

The committee was asked by the EPA to look at the available data on both the genetic and somatic effects of ionising radiation. With regard to the first of these, the committee says that it came broadly to the same conclusions as that reached in the earlier report, namely that one rem of parental exposure throughout the adult population would lead to an increase of between five and 75 additional genetic disorders (over a cur-

rent figure of about 100,000) per million live-born offspring.

“The first BEIR report used mice data exclusively to estimate the incidence of chromosomal aberrations. We have had a limited amount of human and monkey data that has since become available, and have come up with figures that are not significantly different,” said Dr Dean Parker, chairman of the genetic effects subcommittee.

By far the largest section of the report—a single chapter taking up over 500 of the report's 850 pages—is devoted to assessing the somatic effects of high- and low-LET (linear energy transfer) radiation, the first including radiation such as that emitted by alpha particles, the second by X-rays and gamma-rays.

The report says that over the past few years, new studies have modified some of the earlier views about radiation induced cancer in humans. For example, solid tumours—in particular breast, thyroid and lung cancer—were of greater significance than leukaemia in assessing the risks of whole body exposure, and partly as a result of this, it was now felt that the total cancer risk was greater for women than for men. In addition, there was increasing evidence that age is a major factor in cancer risk related to radiation exposure.

Addressing the effects of low levels of radiation—of the order of tens or hundreds of millirads—to which the general public might be exposed in addition to the natural background, the committee says that at present there is no way of telling whether this exposure is detrimental, and that in any case the somatic effects would be masked by environmental or other factors.

Given the lack of data, it says that “for most radiation-induced cancers, the possibility of a linear no-threshold dose-effect relationship cannot be excluded”, adding that, for low doses, the linear hypothesis is “consistent with plausible carcinogenic mechanisms at the level of the single cell.”

However, it points out that the linear hypothesis probably overestimates the risk from low-LET radiation (such as X-rays and gamma-rays), but may underestimate the effect at low levels of high-LET radiation, such as alpha-particles.

Referring to those who receive occupational exposure of between 0.5 and 5 rems a year, the latter now being the recommended maximum in the US, the committee says that “a discernible carcinogenic effect could be manifest” at these levels of exposure. (The average

whole-body dose rate to the 30,000 people currently working in the US nuclear industry is between 0.6 and 0.8 rems a year).

Applying the linear hypothesis to a variety of available data—in particular that obtained from survivors of the atomic bombs dropped on Nagasaki and Hiroshima—the committee estimates a lifetime excess of cancer incidence caused by low-LET radiation of between 192 and 756 cases per rad per million males receiving a single exposure, and between 344 and 1,031 cases for females exposed under the same conditions.

The number of excess fatal cancers was estimated as between 70 and 353 per million per rad per year for single exposure, and between 68 and 293 for continuous cumulative exposure. In comparison, a report published by the United Nations Scientific Committee on the Effect of Atomic Radiation in 1977 estimated an excess of fatal cancers of 100 per rad per million for both categories (the higher BEIR estimates are largely the result of assuming a cancer risk that increases with age).

The legitimacy of these figures is questioned, however, by a number of members of the somatic effects subcommittee. Following what are described as “unresolvable differences”, five have signed a minority report stating that the risk estimated given in the report for specific organs—many of which were derived from the Japanese studies—were excessive, and that these figures “might be used to justify radiation exposure but not to indict it.”

Dr Rossi claimed that there was much radiobiological and epidemiological data indicating that the linear hypothesis led to substantial overestimates of radiation risk. “There is therefore a danger in a report which includes any evidence of an increase when I feel that the risks are nowhere as high as those in the report”, he said.

Dr Radford agreed with Dr Rossi that the general alarm about radiation dangers associated with nuclear power were not entirely justified. “It is a risk, but it is not the end of the world by any means. People do things to themselves that carry a much greater chance of leading to cancer than the effects of nuclear facilities,” he said.

However, he defended the committee's decision to support the linear hypothesis for low level radiation, and argued that the issues raised about the interpretation of scarce scientific data were sufficiently important for the scientific community at large to become



involved in the debate. "Ionising radiation is a prototype of other environmental agents which may be carcinogenic, even at low levels, so a resolution of this matter is therefore important" he said.

In a separate study published earlier in the week, another NAS study group looking at the risks associated with

nuclear power concluded that according to evidence in the scientific literature, exposure from routine operations of the nuclear industry increased the cancer risk of the most-exposed members of the public by 0.1%.

If the nuclear industry was to expand along currently expected lines, there would be between 165 and 255

extra cancer deaths attributable to nuclear power operations up to the year 2000. The academy report says that production of an equivalent amount of electricity with coal-fired power stations would result in an estimated number of more than 3,000 associated deaths.

David Dickson

## Weapons laboratories 'should retain links with university'

AN advisory committee last week recommended to the US Energy Secretary, James Schlesinger, that the University of California should continue to manage two laboratories which carry out the bulk of US research into nuclear weapons. However, the committee says that the university needs to improve the effectiveness of the "trusteeship" it has for the two laboratories, the Lawrence Livermore Laboratory outside San Francisco and the Los Alamos Scientific Laboratory near Santa Fe, New Mexico.

The committee has also advised the department to make a close study of alternative ways of managing the laboratories—in particular it suggests a private, non-profit corporation—if opposition within the university to the current arrangement should grow to a point which makes it "undesirable or impossible for the university to continue the present relationship".

The university's links with the two laboratories, whose activities grew out of wartime research but recently expanded into energy and environmental research, has long been a source of controversy. Faculty and students have attacked the arrangement, some on the basis that the university's responsibility for classified research is unethical, others objecting more specifically to the weapons research.

The current arrangement under which the university manages the two laboratories for the Department of Energy comes up for review next year. Last December, Mr Schlesinger asked a working group of the Energy Research Advisory Board (ERAB) to review the current relationship and recommend how it should evolve "to best serve the needs of the nation and the laboratories". In its report, which was forwarded to Mr Schlesinger by the board last week, the working group says that the factors which have made the university beneficial to the country and the laboratories are "lasting and fundamental", and that the relationship should therefore be continued and improved, even though the university needed to discharge more fully its trusteeship. Referring to what it calls a small but vocal opposition to continued nuclear weapons R & D in any form, it says that protesters often complicate the laboratories' already

difficult public relations by instigating press coverage of "peripheral issues". "The laboratories need support in this area which the university could help provide" the working group says.

However it adds that more pressures may develop both within and outside the university, making it impossible for the university to continue the present relationship. "The DOE must be prepared for such possibilities, even though we believe them to be remote," the working group says.

The report generated controversy when it was discussed at a meeting of the full advisory board in Washington last Thursday. Mr Tom Cochran of the Natural Resources

Defense Council, said he felt the working group had not dealt fully with the criticisms that had been made of the current arrangements, and that it had narrowed its focus by discussing merely the university's ability and apparent willingness to manage the laboratories, ignoring broader issues.

After considerable debate, the board agreed by 13 votes to 4 to forward the working group's report to Secretary Schlesinger, with a covering note from the chairman, Dr Saul Buchsbaum, explaining why the working group had interpreted its brief in the way that it had. A minority report is being prepared by dissenting members.

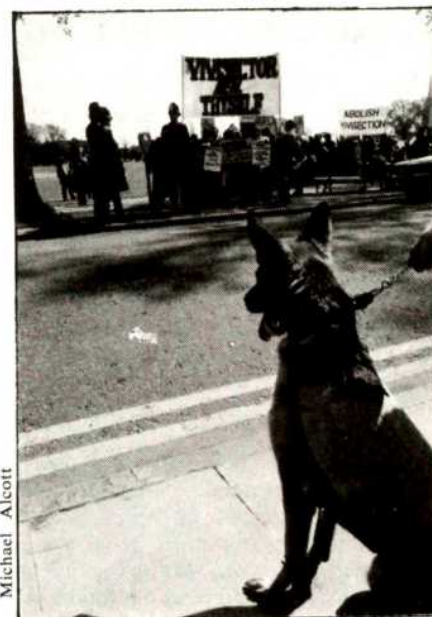
David Dickson

## Anti-vivisection demo hits Cambridge

LAST weekend about three hundred people gathered in Cambridge—the place described by their leaders as the Mecca of biological research in the UK—to voice their disapproval of the use of animals in scientific experiments. For more than an hour they marched through the streets shouting their protests at empty laboratories and Saturday afternoon shoppers. They carried placards bearing slogans claiming "Cambridge scientists torture animals" and "your taxes pay for laboratory sadists to mutilate animals with no anaesthesia". The odd one called for the banning of the seal hunt and an end to factory farming.

The demonstration, which had attracted protestors from as far afield as Lancashire, Gloucestershire and London, was organised by Jean Pink of Animal Aid, an organisation concerned with the rights of animals, whose aim is to achieve the abolition of all animal experiments. "Vivisection is the ultimate in animal abuse", said Mrs Pink. "We demand the total abolition of the use of animals in laboratories".

The protest also included the delivery of a letter to the Vice-Chancellor of Cambridge University expressing the group's concern and a talk by Hans Ruesch from Switzerland, whose book *Slaughter of the Innocents* has recently been published. There is total ignorance about vivisection said Hans Ruesch. He claimed



that few know of the "complete and utter uselessness of medical research through experimentation". Movements to seek the abolition of animal experiments had recently started up in several countries, he said, in particular West Germany and Italy, and also Australia.

The meeting ended with a request that the new Home Secretary, Mr William Whitelaw, who has just taken up his appointment after the election of a Conservative government, should be bombarded with letters as soon as he gets to his new office.

Judy Redfearn



# Soviet 'mental illness' spreads to Czechoslovakia

Dr Snezhnevskii, of the Serbskii Institute of Forensic Psychiatry in Moscow, is well-known as the man who provided a theoretical basis for the practice of interning dissidents in mental hospitals. According to Dr Eva Dubska who left Czechoslovakia in 1977, at the time of her departure, Snezhnevskii's works were gaining support among certain sections of the Czechoslovak psychiatric establishment. A case history has now reached *Nature* which suggests that the Czechoslovak political establishment is prepared to add its weight to them.

According to Snezhnevskii, "all psychological illness is a form of schizophrenia. Further, he identifies a number of subtypes and symptoms not recognised abroad. These include "sluggish schizophrenia" (with no perceptible symptoms), "delusions of reformism", "a mania for protest" and the like. In the case of Augustin Navratil a 45-year-old Czech recently committed to the mental hospital in Kromeriz, there are disturbingly clear indications that the same diagnostic procedures have been used.

Navratil, who served as a local councillor for the revived People's Party under the Dubcek regime and who has since worked as a railway signal-box guard, was charged in January 1978 with slandering the state. (He had been collecting signatures for one of the many civil rights petitions which sprang up in the wake of the Charter-77 movement.)

After considerable bureaucratic delays, he was sent to the local mental

hospital for examination, and found unfit to stand trial; accordingly he was formally committed for treatment. The diagnosis includes such Snezhnevskii type expressions as "hysterical self-stylization towards the ideal of a strong leading personality and with a strong moral responsibility which the subject understands as 'fidelity to his principles' and an inability to adapt to an adequate view of social reality". Further "by analogy with the fate of past personalities the subject thinks that 'for the truth one must logically suffer'."

At times the report becomes near ludicrous, quoting IQ figures as evidence of an unbalanced personality, urging treatment although admitting that Navratil cannot be brought to change his views. However, it is suggested, it may be possible to make him conform at least outwardly.

It is, perhaps, this Kafka-esque cynicism which is the most alarming aspect of the case. Reading the report, one gains the impression of a trained scientist desperately trying to adduce some kind of substantiation for a diagnosis already determined. □



"I'm afraid, doctor prosecutor, you are suffering from hysterical self-stylisation as a psychologist with an inability to adapt to an adequate view of humanity"

## French stress scientific side of detente in space

Mr Brezhnev's invitation to France to supply a crew-member for a joint manned space-mission with the Soviet Union did not come as a complete surprise to the Centre Nationale d'Etudes Spatiales (CNES). Shortly before President Giscard d'Estaing left for his "official working visit" to the Soviet Union, the chairman of CNES, Professor Hubert Curien, said that CNES was considering the possibility of Franco-Soviet cooperation in materials-processing in conditions of weightlessness.

CNES welcomed Mr Brezhnev's proposal in principle. They are not, however, interested in France simply being another country to have one of its citizens in orbit. "We are a scientific group", a CNES spokesman told *Nature*. "Such cooperation could be quite interesting, but we want to know what programme or programmes would be involved. We have a lot of interesting programmes in hand, and if the cooperation comes to pass, it would probably be one of the people in charge of one of these programmes who would take part."

No date has yet been even tentatively set for such a mission, nor have any details been worked out. But if CNES stand by their wish that "he would be an experimenter rather than a pilot", a significant change in Soviet procedure would be involved. Since the

three crew members of Soyuz-11 died tragically during re-entry, Soviet cosmonauts have always worn space-suits aboard the Soyuz transport vessels. The extra bulk means that only two cosmonauts can be launched in one craft. Although one is officially designated the pilot, the other is trained to be able to take over the controls in an emergency. CNES, however, would appear to prefer their representative to be essentially a passenger, ferried up to an orbital station where he would then proceed with his experiments.

Although CNES, as scientists, deprecate the publicity value of participation in the Soviet space programme, both the French and the Soviet governments seem to place great stress on it as what Mr Brezhnev last week called a "barometer of detente". France has occupied the leading position among non-Comecon countries in bilateral space cooperation with the Soviet Union.

The first general cooperation programme to include space was signed in 1966, and was presumably intended by the Soviet Union to encourage de Gaulle in his attempts to detach France from the US and NATO. Although French foreign policy has changed under succeeding presidents, the general world climate of detente and the impetus which the Franco-Soviet

space programme had already built up ensured its fruitful continuation.

France has, accordingly, taken part in a number of joint projects with the Soviet Union, including a laser-reflector experiment to determine accurately the distance to the Moon, a study of the atmosphere of Venus, investigation of solar radiation from Mars probes, studies of the magnetosphere, solar neutrons and gamma rays, and a programme of simultaneous rocket launches to study the *Aurorae borealis* and *australis*.

At present, aboard Salyut-6, cosmonauts Vladimir Lyakhov and Valerii Ryumin are responsible for a France-Soviet materials-science experiment to synthesise magnetic alloys from materials which do not give rise to magnetic compounds in conditions of Earth gravity. This type of experiment will certainly be in the CNES planners' minds when they work out their programme for their "experimenter". Before he dons his space-suit, however, one important point remains to be settled by that branch of the Academie Francaise which regulates neologisms and loan-words. Is it the nationality of the crew-member or the registration of his craft which determines his designation? In other words, will he be officially described as an astronaut or a cosmonaut? □

**Carter urges expanded nuclear power programme:** President Carter has told members of the US Congress that he strongly supports an expanded nuclear power programme, and that despite his continued opposition to the liquid metal fast breeder reactor at Clinch River, Tennessee, he is still in favour of the development of a more advanced fast breeder. "I want to emphasise that my opposition to the CRFBR does not imply my opposition to breeder reactors in general or to nuclear power", the President wrote in a letter to Congressional leaders asking them to support his attempts to halt the Clinch River project. "We need to pursue a vigorous programme of breeder reactor research and development so that this option can be commercially available to us if and when we need it." The President's remarks became publicly known two days before a mass anti-nuclear demonstration was held in Washington.

**Official BNFL response on Windscale leak:** Sir John Hill, chairman of British Nuclear Fuels Ltd, has responded to government enquiries about the highly active Windscale leak first reported on 18 March. In a letter to Tony Benn, outgoing Energy Secretary, Hill outlined the history and current status of the leak. High activity of 200 millicuries per litre was first recorded from borehole number 63 in November after four previous samplings done earlier in the year showed a lower activity of 0.2 mCi/l. This lower activity "did not cause particular concern" says Sir John because it was consistent with contamination due to a spill occurring twenty-five years ago. By March, investigations showed that the new activity was consistent with activity discovered in an out-of-use steel-lined sump located in an annexe where buffer storage tanks are housed. The route from the sump to the borehole is still unknown, however. The letter states that laboratory tests show that the "mean" migration rates of  $^{137}\text{Cs}$  and  $^{90}\text{Sr}$  in soil are about 1 metre in "several hundred days" so that activity will have decayed "to an insignificant level before the site perimeter is reached".

**National Cancer Institute criticised over carcinogen testing:** The US National Cancer Institute in Bethesda, Maryland, has been strongly criticised by the General Accounting Office, the investigating arm of the US Congress, for deficiencies in the managements of its programmes for testing substances for possible carcinogenicity. In a report published last week, the GAO says that in particular the Institute failed to spot serious deficiencies in testing programmes carried out on a contract basis by private research companies. The GAO said that many of these companies used unacceptable procedures to test chemicals, and that many test results had had to be thrown out by the Institute, at a cost of millions of dollars. Another report published by the GAO has criticised the lack of sufficient training in professional ethics given to employees of the National Science Foundation, particularly over activities relating to dealings with possible future employers. The GAO says that in discussions with programme managers in the NSF, investigators found that it was not unusual to be asked for an interview with an organisation for future employment while involved in reviewing grant applications or monitoring ongoing work for that organisation.

**DHSS proposes increased job security for research staff:** Professor A. J. Buller, Chief Scientist at the UK Department of Health and Social Security, has proposed a new scheme to save the jobs of scientists faced with unit closures. Under the scheme participating directors of DHSS-supported units will consider researchers who are made

redundant by unit closures for any vacancies that arise—before they invite applications from other candidates. Directors of units are under no obligation to hire anyone they consider to be unsuitable. In addition the DHSS will make extra posts available to directors pending vacancies in their normal complements. The DHSS will also pay for researchers' removal costs. But researchers will be expected to move to any part of the country and if they refuse, "even if there is only one offer they will be made redundant". The proposal has been circulated to the 34 DHSS-supported units and will be put into operation when 20 units have accepted.

**Australian trade unions act to stop proliferation:** Mr Cliff Dolan, vice-president of the Australian Council of Trade Unions, announced last week that three major trade unions would withdraw their labour to halt two new uranium projects in northern Australia. Citing the dangers of proliferation, waste disposal and health hazards, the ACTU has opposed the mining and export of Australia's considerable uranium reserves estimated to be 20% of the Western world's supply. The Amalgamated Metal Workers and Shipwrights Union, the Australian Railways Union and the Electrical Trades Union have agreed at recent meetings to follow the ACTU's recommendation. The unions will stop manufacture of mine equipment and its transport to mining sites.

**Sweden and China to cooperate:** The Royal Swedish Academy of Sciences and China's Academia Sinica have signed an agreement on cooperation in basic sciences. The agreement, signed in Peking during a recent visit by Professor Carl Gustaf Bernard, secretary of the Swedish Academy, comes into force on 1 July and provides for mutual exchanges of scholars. The Swedish Academy of Engineering Sciences has signed a similar agreement with the Chinese. In a discussion of Chinese science policy, Vice-President Fang Yi told Bernard that he was trying to restore the damage done to basic science by the Gang of Four. Training scientists in the West was a good way of tackling the problem. Several hundred scientists will probably visit Sweden under the new agreement, beginning with ten interested in methodology in basic research. Another area in which the Chinese want Swedish help is geology. Developing their natural resources in one way of paying for their modernisation programme. In response to a question on environmental pollution, the Vice-President replied that in a period of enormous expansion, it was more important to build up the industries than to confine their pollution.

#### **India's new radio telescope operational soon:**

By the end of this year India's new aperture synthesis radio telescope will be fully operational. It is the latest addition to the Tata Institute of Fundamental Research's (TIFR) Radio Astronomy Centre at Ootacamund. Like the previous Ooty Steerable Radio Telescope, which was commissioned in 1970, its design and construction is completely Indian. Its resolution will be equivalent to that of a 4 km diameter parabolic dish. The new telescope is an assembly of seven antennae which will be linked to the main Ooty Steerable Radio Telescope. The total estimated cost of the additional antennae and associated equipment is 4 million rupees (£180,000), an amount which makes it one of the cheapest aperture-synthesis radio telescopes.

*From Dilip M. Salwi in New Delhi*





# Energy search comes down to earth

David Dickson reports on US hopes of extracting heat directly from the earth's crust

Hot dry rock, its supporters believe, offers one of the few practical, medium-term alternatives to fossil or nuclear sources of energy with little of the environmental problems or shortages associated with either. If it can be tapped efficiently, the potential energy available from the heat stored in the Earth's crust is enormous. It has been estimated that the energy supply for the US for a whole year is contained in a 50 cubic mile block of hot granite.

"We also think that it is the cleanest source of energy on the horizon," says Mr Greg Nunz, recently appointed manager of the Department of Energy's National Hot Dry Rock (HDR) Program at the Los Alamos Scientific Laboratory in northern New Mexico, where research into possible extraction techniques has been going on since the early 1970s.

At present, the most traditional and widely used method of extracting geothermal heat is through tapping underground reservoirs of hot water. The Los Alamos scientists, however, are developing ways of extracting heat from rock which is usually a result of radioactive decay, or of molten magma below the Earth's crust. The technique is to pump water down from the surface into a fracture that has been artificially created in impermeable rock. The water absorbs the heat from the rock, and is pumped back up to the surface again under sufficient pressure to prevent it from boiling.

"The concept is really very simple: we are literally trying to 'mine' the heat by creating a heat-exchanger underground", says Mr Nunz, pointing out that HDR techniques are therefore more flexible than those which require the existence of natural hydrothermal reservoirs.

Soon after discussions began at Los Alamos about the potential of HDR, field investigations were carried out into the high thermal gradient characteristics of the nearby Jemez Plateau, site of the third largest extinct volcano in the world. A preliminary test hole was drilled to a depth of 785 metres, where the temperature of the surrounding rock was found to be 100 °C. A second hole following two miles away at a site known as Fenton Hill. This found a temperature of 197 °C at a depth of 2,929 metres.



Steam is released at the hot dry rock facility, Los Alamos

The next stage was to pump water under pressure down the drill-hole, opening up plate-shaped cavities—the largest having a radius of 215 metres—at the bottom. A further hole was then drilled from the same site in the hope that it would intersect the largest cavity and provide a channel for hot water to be returned to the surface. Uncertainty about the exact shape of the cavity, however, meant that the new hole failed to make the intersection. Mr Nunz explains that "the instrumentation of the time was not up to the job".

The next two years were spent developing techniques to get a better understanding of the geometry of well-bores and of fractures. In early 1977, the first of the Fenton Hill holes was re-drilled, this time successfully intersecting a fracture made at the base of the second. And late in the year the first circulation of water took place, using a heat-exchanger at the surface to disperse the heat brought up.

Tests carried out so far on the completed system have been a "tremendous success", says Mr Nunz. For example, during an initial 20-hour run the temperature of the water reaching the surface rose by 130 °C. Water quality was good, with a low concentration of minerals that could have clogged up the system.

This was followed by a 75-day test at the beginning of last year, during which the amount of energy extracted rose to about 5000 kW. The amount of water lost to the surrounding rock stabilised at no more than 1.5% of the circulation rate.

The success of this trial system led the Department of Energy to announce last autumn that it was setting up a national Hot Dry Rock Geothermal Energy Program, to be managed by the Los Alamos Scientific Laboratory—itsself run by the University of California for the department—with the overall aim of determining the potential of HDR as a significant energy source, and if possible of providing a basis for its commercial development.

The scientists at Los Alamos are already convinced that energy produced in this way—at least that which

can be used for heating—is potentially competitive in price with other more conventional sources of energy, and is likely to become increasingly attractive as the price of the other sources continues to rise. A recent study carried out with economists from the University of New Mexico, for example, reached the conclusion that in areas where the geothermal gradient is 40 °C or more per kilometre depth, electricity produced by HDR techniques would be competitive with other sources producing energy at 3 cents a kilowatt-hour.

The scientists also claim, that, so far, they have not been able to detect a significant environmental impact from the work on the Fenton Hill site. There has been virtually no disruption of local ecosystems, neither has the drilling caused any measurable seismic activity.

The next step in the national programme is to see if the results achieved at Los Alamos can be repeated elsewhere. Already three of the 100-mile square sites in different parts of the US are being examined as possible candidates for a second drilling programme. Meanwhile back at Fenton Hill, work has already started on a deeper hole, drilling to temperatures around 260 °C, which, it is hoped, will be able to demonstrate the commercial potential of HDR to interested utility companies. If successful, this could lead to a 50 kW experimental plant, sufficient to provide the electricity needs of 5,000 people.

One problem that the programme managers face, however, is gaining public recognition of the potential of HDR. "Right now, public perception of the potential is almost zero. And we are planning to have a contractor look at the problem and decide the most cost-effective way to do some public education," says Mr Nunz.

Among those to whom the message will be directed are likely to be members of Congress. So far federal support for the HDR programme has been extremely modest compared to the amount spent on research on other energy sources. Funds for the first few years' research came from the Laboratory's discretionary money, and



over a seven-year period the whole programme has cost under \$40 million.

Already other countries, particularly those with favourably geological conditions, have shown close interest in the programme. Once a year the programme managers hold a formal seminar in Sante Fe to discuss the progress of the project, attended by scien-

tists from many parts of the world. Both the Japanese and West German governments have offered to provide funding for the programme.

The federal programme is now focused on 1986, when a decision will be taken on whether to move the whole programme up to a near commercial scale.

At Los Alamos, they are confident that these investigations will be successful. "I don't think that the world has discovered hot dry rocks yet. But we see it as the only major alternate energy source that can be developed in this century, and do not understand why the powers that be are not more excited about it," says Mr Nunz. □

## Harrisburg shakes up Sweden's nuclear war of words

THE Harrisburg reactor accident hit the Swedish nuclear energy debate like a karate chop. The Social Democrats, long supporters of Sweden's nuclear development and opposed to a referendum on it, announced that the accident was serious enough to make them change their basic judgements: there should be a Swedish committee of enquiry into Harrisburg and a referendum should be held. The Liberal party government changed with them and declared that no more reactors should be loaded until after the referendum. And an opinion poll taken after the accident shows a dramatic anti-nuclear swing: 53% of people asked said that they would vote against nuclear power in a referendum and only 26% said they would vote for. In January, the same question produced 43% against and 41% for. On the face of it, then, Sweden's nuclear future is wide open again. Or is it?

The seven years of Sweden's public nuclear debate has shown that facts enter politics only to the degree convenient for either side. More facts are being gathered now: a party of Swedes and Danes left this week for Harrisburg, and, on the domestic front, a working group is about to investigate Good Friday's accident at the Barsebäck 1 plant, within sight of Copenhagen. In this accident—"the worst in Sweden so far", according to a spokesman at the Nuclear Power Inspectorate—the generator exploded, throwing out metal projectiles (including a two-ton steel capsule) which did not, however, penetrate into the reactor itself. But it is hardly likely that any of the facts uncovered by these groups will be treated any differently from those already paraded. The referendum, to be held next spring, will be advisory—but its 'advice' will no doubt be used only so far as the politicians find convenient.

The last act of last year's three-party coalition government was to refuse the nuclear industry's application for the loading of the Ringhals 3 and Forsmark 1 reactors, on the grounds that the industry had not shown the existence of a sufficiently large rock formation with appropriate characteristics for final disposal of waste. The government said that, if the industry found rock they

considered more suitable, and if it is approved by the Nuclear Power Inspectorate, the government would give permission to load the reactors. After the coalition fell, and the pro-nuclear Liberal party government took over, the industry duly made more inspections on Sternö, near Karlshamn in southern Sweden, and made a new application.

The Inspectorate decided to appoint a group of eight independent geologists (six from Sweden, the others from Norway and Finland) to assess the industry's report, giving the Inspectorate's Board a factual base for its recommendation to the government. The new government announced that it would be bound by the Inspectorate's recommendation—and that it was confident that the Inspectorate would be able to recommend that the loading should go ahead.

### Rock unsuitable

Seven of the eight geologists found that the geological properties of the Sternö rock were such that it "cannot be used for the storage proposed by the nuclear industry". They reported: "The holes drilled (by the industry) are too few and drilled in such directions that, even if they had all shown fracture-free, homogenous rock, they would not have sufficed to show unambiguously that the rock fulfils the set conditions." The group found fractures not discovered by the industry, and these could reduce the area usable for storage from the desired 1 km<sup>2</sup> to 0.3 km<sup>2</sup>. Although storage requires homogenous rock, the investigated area contained three different types of rock, and the consequences of this had not been sufficiently investigated. The group maintained that it is impossible, at present, to determine the permeability of the rock—yet the nuclear industry's safety analysis depends on this parameter.

But the Inspectorate's Board did not recommend that the loading be stopped. In a masterly formulation, it told the government that the geological qualities of the rock must be seen in the context of *all* the barriers designed to prevent leakage of waste including vitrification and encapsulation. "The

Inspectorate does not maintain that each one of these barriers must give that degree of protection assumed by the nuclear industry in order for the final result to be regarded as wholly secure. . . . The importance of the demands made on the geological barrier should not be exaggerated . . . if the other barriers work satisfactorily." The question of whether the industry had shown that the rock met geological standards for deposition of nuclear waste was neatly sidestepped. The Board said that it was not unanimous on the meaning of the word "show", and therefore could not say whether the standards had been met or not.

"This has been such a political affair that science has been totally overridden", says Dr Arne Wesslén, one of the eight geologists. "It is regrettable that a regulatory authority can appoint a group of experts who give such a consistent opinion, and then ignore it for political reasons." Dr Bengt Åberg, another of the geologists, agrees. "We did the work scientifically. The Inspectorate tried to find ways around certain parts of our report", he says. And a spokesman at the Inspectorate admits, "We found ourselves under intense political pressure".

Ironically, all the geologists think that it should be possible to find rock in Sweden with the desired characteristics. "Sternö was not chosen for geological reasons", says Dr Åberg. "The industry has had to rely on areas it owned or where it was given permission to drill. On Sternö, no holes could be drilled outside the power station fence! We should have a law allowing drilling to be done in geologically-likely areas."

The day after the Inspectorate's Board met, the accident happened at Harrisburg. In the aftermath of that, with the ban on loading more reactors until after the referendum, the Board's verbal acrobatics proved to have been unnecessary—for the time being. Politicians' energies are now concentrated on the fight over the wording of the referendum question, and bitter fights lie ahead. In the meantime, as September's general election looms, nuclear power is not likely to be forgotten.

Wendy Barnaby



**UNCSTD '79:** This is the first of a series of articles leading up to the opening of the United Nations Conference on Science and Technology for Development (UNCSTD) in Vienna on 20 August. So much hot air has risen over the nature of the conference itself that the real problems facing science and science planning in the Third World have been forgotten. So we sent a team of writers to the grass roots. We asked Dr Hanlon

to look at the problems, on the ground, of an African country. He chose Ghana. We asked Anil Agarwal, a respected Indian science journalist now working in London, to visit India to report on the changing shape of science under the Janata government. And we have granted writing fellowships to scientists to visit and report on affairs in South America. We shall also have reports on South-East Asia and the Middle East.

## Ghana: struggling for scientific independence

"TWELVE years ago I came back with a PhD in magnetohydrodynamics. I tried to work here, but there was no point. It was totally useless in Ghana." Now K. O. Kessey is professor of mechanical engineering at the University of Science and Technology (UST), working in refrigeration. "Twelve years ago, I couldn't imagine me doing earthly things—I was highly mathematically inclined. But you must use your knowledge and experience in a new situation. Students come back from abroad and they are helpless and hopeless. They say 'Oh, I don't have the equipment to continue my work'. So they do nothing. But it's just an excuse."

Dr Dwuma-Badu, head of the UST pharmaceutical chemistry department, and one of many working on herbal medicine, said: "With the equipment here, we cannot challenge pharmacy schools in developed countries with synthesising new compounds. So we work with Mother Nature to find what is there."

These comments reflect a growing change in Ghana. Science there used to be closely modelled on the British pattern; but as more graduates returned from studying abroad they began to question the relevance of their training—and of Western science in general. Meanwhile, the government, which had previously ignored science, began to ask what it was getting for its money. Both sides began to realise that Western science had simply been transplanted and allowed to grow in isolation, producing neither good science nor anything useful for the country.

As a result, the government set up a committee to review the Council for Scientific and Industrial Research (CSIR), and after its highly critical report, CSIR sharply changed direction. The Five Year Plan published two years ago was also the first Ghanaian development plan to have a section on science and technology.

As Dr M. N. B. Ayiku, head of CSIR's Industrial Research Institute explains, "there is only one science—only one set of laws of nature. But in a particular society, a certain type of science is emphasised. There is bound to be an accent based on our own problems. We must start small and

First World universities still set the pace for science in Africa. But the resulting research is often irrelevant

### Report by Joseph Hanlon

solve the little problems first. But as you solve the little ones, you unearth the big ones." And that brings new areas of basic science into view.

Professor Albert N. Tackie, head of CSIR, points to the other side: no one else will solve Ghana's problems—such as malaria. "If we want to tackle our diseases, we will have to do the research." Similarly, he points to building, where Ghana still relies on imported cement when it should be concentrating on local bricks and timber. The latter requires the study of another problem that is much less serious in temperate zones and thus little researched—termites and other insect pests.

Basic research—now underway on several Ghanaian problems, may have application elsewhere, for example:

- At UST, the chemistry and biology departments are working on bacteria to leach gold ore from the sulphur in which it is embedded.
- At the University of Ghana geologists are studying an earthquake fault which runs through Accra. It has not been active until recently, and its activity is not explained by current theories, so it is of general geological interest. The research also has practical value for Accra, where 16 people were killed in the 1939 earthquake.
- The Food Research Institute is looking into the preservation of kenky, fermenting corn meal, which must be sold and eaten in just a few days before it becomes too sour. The Institute is experimenting with dehydration to improve storage and distribution. Little work has been done elsewhere on drying fermented doughs, because they are rarely eaten in developed countries.
- Ghana is well-advanced in the study of herbal medicines (the subject of a later article).

Not all of Ghana's scientists have made the transition; it has been hard even for those who support the changed policy. J. Maud Kordylas, head of the Food Research Institute,

talked of her own research. She is now working on the use of the winged bean as a weaning food. Nutrition studies have shown a definite need for a supplemental weaning food, but peasants cannot afford to buy commercial ones. The winged bean can be grown locally and is an important source of the amino acid methionine. Kordylas's work on processing the bean is very different from what she was doing before. "I worked on vitamin A metabolism with respect to cholesterol at the London School of Hygiene and Tropical Medicine. I see high blood pressure in Ghanaians and I know they eat a lot of palm oil which is high in vitamin A, so I know it would be useful. And that sort of work gives me more satisfaction as a person. But we have many nutritional problems. In practice, the winged bean is a more useful project for Ghana."

Postgraduate studies abroad remain a necessity, but Ghana now scrutinizes these more closely. CSIR sends many of its research officers abroad for MScs and PhDs, and four years ago, it began vetting their research projects to make sure they are relevant, according to Tackie. Nicholas Darkwa, for example, has recently returned from North Carolina University with a PhD in pulp and paper science. His research topic was making paper from the stalks of plantain, a banana-like plant very common in West Africa, and CSIR actually shipped plantain stalks to him in the US for his work. Another CSIR researcher is studying making paper from local hardwood trees as part of his work in the USSR. Tackie hopes that the two will form the scientific basis of a Ghanaian paper industry.

The other side of education abroad is the brain drain, both short and long term. Foreign countries may subsidise Third World students, but they extract a high price—encouraging the brightest to remain abroad. UST vice-chancellor E. Bamfo Kwakye comments "to send your best students out means that you neglect your own postgraduate activities. Furthermore, it costs 24,000 cedis a year to keep a postgraduate student in the US—we pay a professor less than half that. Finally, we have severe staff shortages." So UST has changed its policy: fewer students are sent abroad, and an effort is being



made for joint MSc programmes, in which the student returns to UST to finish his or her research.

Although the new policies of UST, CSIR, and others are working well in some areas, in others they are not. UST has a joint chemistry MSc programme with a foreign university which simply allowed UST students to enrol there for a PhD rather than return to Ghana, defeating the whole point of the programme. A physicist told me he was working on electron transport in thin manganese films, a project of no apparent relevance to Ghana, because it was the area of a visiting British professor. Manganese may not be useful for Ghana, "but for someone who wants to get his PhD and get promoted, it is useful," commented the physicist honestly. Promotion is always a bugbear in science, and it is a special problem in Ghana, both because the system is very rigid and because it has failed to take into account the new attitudes toward research. "People are employed on the basis of being world class scientists who are employable in Britain or the US, not on whether they can solve local problems," declared M. D. Swain, a Ghana University botanist.

Promotion at CSIR, for example, is based on four things: a list of publica-

tions, reports on those publications by outside assessors who are primarily foreign, annual assessments, and supervisor's comments. There is little consideration of work that may have been highly useful but has not led to publications. Ayiku complained: "If I want promotion in CSIR, my *curriculum vitae* is sent to Germany or England, where I am judged by European standards." CSIR claims to have changed its policy somewhat, using a few local assessors for papers, for example. But few people, inside or outside CSIR, seem aware of the change.

In practice, the universities are just as bad. "It shouldn't be the *sine qua non* for promotion to be published in prestige Western journals," admits Kwakye—but it still is.

Often, because of rigidity, places simply do not get filled. Several UST departments do not have professors, even though they are being run perfectly well by acting heads who only lack the paper qualifications. One University of Ghana department has four of its 11 senior positions vacant. Yet, when a highly competent man applied, he was rejected—because he had failed his first year of university, even though he repeated it successfully and has since done well.

There are few local journals and they are rarely published because of lack of foreign exchange, so publication means publication in a foreign journal. One botanist considered looking at sour grass, a weed first noticed in Africa only two years ago and now slowly spreading out from Accra. It would have made a useful piece of scientific work, ideal for a local journal; but lacking international interest, it would not qualify for a foreign journal. So the work was not done.

Another adverse factor is the lack of an outlet for the results of relevant research. Scientists complain that, despite the lip service paid in the Five Year Plan, research is still not taken seriously. Ghana's national paper for UNCTSD, written by Tackie, complains that "the major scientific and technological institutions such as CSIR and the National Standards Board are not involved in investment decisions . . . development planning is entrusted almost exclusively to economists. . . . There is no effective accompanying technological evaluation."

There is little industrial take up of ideas generated by researchers, either. UST set up a few small manufacturing units on campus using simple equipment and local materials. The engineering faculty makes pumps and traffic lights and the arts faculty has a ceramics production unit. "Originally, we hoped that entrepreneurs would take these out of our hands, but this has not happened, so we continue to manu-

facture," Kwakye said.

Continued dominance of the economy by foreign companies also reduces the opportunity for local researchers. "The multinationals are so powerful; Ghana is so poor. It is impossible to think how we shall break this hold," comments Kwakye. Much applied and basic research is done outside the country or by outsiders. For example, when off-shore oil explorations began, the international oil companies asked local geologists to give them a basic briefing, and then they brought in their own geologists. So the local geologists gained nothing out of the project except a few small fees.

The scientists themselves are partly to blame. The ecology and geology of Ghana remain poorly studied. Tropical forests are Ghana's second highest foreign exchange earner, yet they are little researched. Tropical ecology is different and much more complex than temperate, and is sure to become a prime research area as the temperate zones are better understood—indeed, much basic science in the future will probably have a tropical bias. But it is not the Ghanaians who are moving into these new areas, but the white expatriots and visitors. Even the research into earthquakes is dominated by people and equipment from London University.

Some change does seem to be taking place in Ghana, however. There has been a realisation, as the UNCTSD paper notes, that "some of the measures taken so far towards the goal of economic independence have paradoxically led to a greater degree of dependence on the industrialised nations." Ayiku notes that Ghana has been buying technology as if it were Coca Cola—to be consumed and thrown away with no understanding of it. As Ghana University geologist Mike Mensah points out: "a developing country that cannot do research will always be an importer of technology."

But, as the UNCTSD report also notes: "The Ghanaian experience suggests that mere growth in the volume of scientific research or the use of advanced sophisticated industrial plants or machinery will not of themselves bring about development. It is essential that scientific research should reflect the true needs of development."

This must be done with the facilities available in Ghana. Ivan Addae-Mensah, a University of Ghana chemist, concludes: "If you want to do high-falutin sophisticated research, you can't get anything done here. But if you actually want to be a scientist here, you must look for the problems around you." □

*Dr Hanlon is a science journalist specialising in Third World affairs.*



Above: Professor Tackie, research council chairman. Below: Professor Kessey with down-to-earth invention, a special carrier for keeping vaccines cold.





# Why eating should carry a government health warning

It is time for scientists to swallow their doubts and press for a proper food policy which will save lives, say **John Rivers** and **Philip Payne** of the Department of Human Nutrition, London School of Hygiene and Tropical Medicine

It is generally agreed that food can kill, but argument persists as to whether anything should be done about it. Government action on diet and health has so far been minimal essentially because argument over the exact risks of eating has dwarfed any attempt at exploring the problems of doing something about them. We believe, however, that the latter issue is not merely as important as the former, but must be resolved first: we must know what kinds of action we might conceivably agree to before it is even worth considering the role of diet in disease.

It is widely agreed, by both nutritionists and the general public, that changes in diet occurring in the past 100 years have at least some role in the increased incidence of a whole range of diseases from dental caries and obesity to cardiovascular disease and cancer. These dietary changes have been many and complex, and major controversies exist as to exactly which are to be held responsible for the increase in diseases of affluence.

Considerable publicity has been given to such controversies, not least by interested organisations in the food industry who feel their own products might be under attack. Understandably such publicity as a rule is aimed at defending the notion that all existing consumption patterns are expressions of free choice, and should be regarded as innocent until proved guilty. This philosophy of waiting for absolute scientific proof attracts much support from the health professions, not least because it implies both the need for much continuing scientific research, and that the professions involved will define and apply their own criteria for what constitutes adequate proof.

Paradoxically the adherence of the health professions to a philosophy of waiting for certainty coexists with a broad professional consensus that would support moderation in eating, a reduction in fat and sugar intakes, and, in consonance with many other fashionable theories about conservation and ecological balance, some degree of reversion to diets with greater proportions of plant foods and fewer refined components. This consensus is clearly only provisional. That is, whilst there is not wide agreement that these changes will ultimately be shown as

sufficient, or indeed necessary to bring about a general improvement in nutrition, they are currently widely accepted as reasonable goals for any individual interested in conserving health. It is, in short, the best advice we can offer at present.

Unfortunately the nutrition profession in the UK has a somewhat vacillating attitude about what to do with its consensual knowledge. There is much pride about the role that nutritionists apparently played in the institution of a nutritionally based food policy during the second world war. There is widespread involvement with organisations like FAO and WHO in attempts to ensure that nutrition is integrated into the development policies of the Third World.

Yet concern for the nutritional problems of our affluent society is not translated into such decisive action. Admittedly individual nutritionists try to persuade, cajole and educate the public, but the profession has, as yet, stopped short of concerted action. It does so on the ground that present knowledge is inadequate. Clearly it is, but insofar as dietary moderation would tend to halt some of the major current trends of dietary change, it represents a policy of conservative caution, an attempt to stand still until the implications of change are better understood. This is a powerful argument to weigh against the elusive ideal of certainty.

It is generally agreed that piecemeal nutrition education usually fails, and some government action is needed. If the government were persuaded to support a strategy for health it would require at least consistent and relevant action by various ministries, and possibly regulations for the control of advertising. Such a sweeping approach might well be effective in changing the overall pattern of consumption, but is resisted on two grounds: it would constitute an unjustified interference with free choice; and the economic costs to agriculture and industry of adapting to new patterns of demand would be unjustifiable in view of the uncertain benefits. We reject both these objections.

In the UK, government intervenes in agriculture in many different ways, and any idea that we have a free market economy in food is a myth. The choice



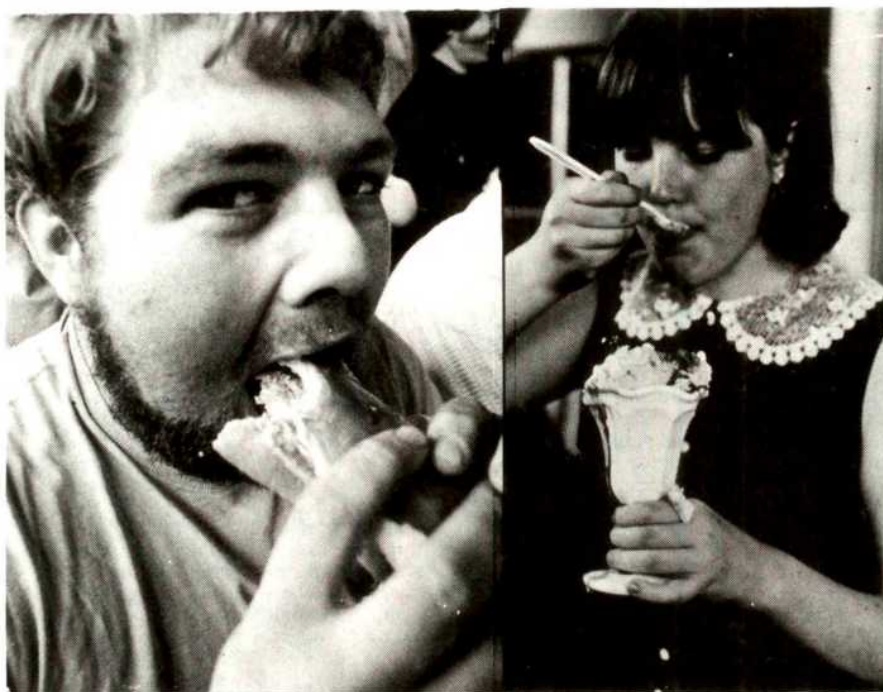
*Choosing can be difficult . . .*

is between agricultural policies that ignore the health of the consumer, and those in which health is one of the considerations used to frame policy. At the moment, health enters only peripherally into, for example, regulations to prevent the adulteration of milk. The central complex of subsidies and support systems that shape agricultural policy in the UK seem to exist for wholly non-nutritional reasons. The subsidy on butter may make political sense, but, in a country where the consensus advice is to reduce dairy fat consumption, does it make nutritional sense?

The incentives for producing fat animals result in masses of fatty carcass waste that is used in the manufacture of cheap convenience foods such as sausages and beefburgers. A health conscious consumer has a difficult balance between cost, time and risk to cope with, and he may feel that choosing a diet he regards as unhealthy is an unavoidable risk. The refusal of the Milk Marketing Board to make reduced fat milks freely available; the absence of regulations requiring the labelling of cheeses with fat value; the widespread use of sugar in processed foods; the high price of wholemeal bread—all these results of government food and agricultural policies, or the marketing practices of virtually uncontrolled oligopolies, seriously impede any individual seeking to follow the sort of dietary advice which most nutritionists, doctors and the UK Department of Health and Social Security offer.

The existence of non-nutritional agricultural policies makes nonsense of the argument that inaction on diet and health should persist until final proof is





... especially when agricultural policies favour convenience foods

obtained. There is no doubt that the current nutritional debate is tentative and incomplete, but if nutritional factors are to be used with political and economic ones to frame agricultural policies, all that need be asked is that the predictions in each area should be equally plausible. To accept political intuition about the benefits of an agricultural policy while making a philosophically impossible absolute proof the criterion for scientific inputs is to emasculate scientific advice to government.

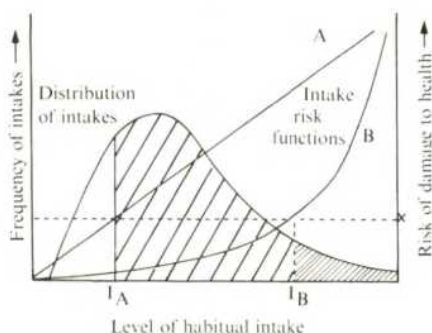
We believe that the health professions should unite in urging now a cautious and conservative basis for a nutritionally oriented food policy. Once they do so, however, there are other challenges to be faced, and it is important to pursue further some of the possibilities which expanding scientific knowledge will raise. It is reasonable to expect a hardening of the consensus view about the adverse health effects of some particular food or nutrient, and questions will then inevitably arise about further legislation, more specific advice or more rigorous action. If we consider now what kinds of scientific evidence would be needed to justify such intervention, nutrition research could be planned so as to contribute most effectively to the social and political processes of policy choice, and hence to health.

Clearly the risks of consumption of a given dietary component vary with the amount consumed. Since there is a wide variation between individuals in the amount of any dietary component that they consume, there will be wide variation in the risk to which they are

exposed. This will be increased further by variations between individuals in their constitutional sensitivity to disease. The benefits derived from a given 'across the board' reduction in intake will also, therefore, be highly variable.

If food and agricultural policies designed to combat disease are to be both effective and equitable it is necessary to define both the intake-risk relationship, and the acceptable level of risk in quantitative terms before it is possible to consider precise costs and benefits of the policy options open. The problem of what constitutes an acceptable level of risk is encountered in legislating against all sorts of hazards and the defining of acceptable levels is not a scientific problem, but a social and political one. However, the problem of intake-risk relationships does require scientific definition, since their nature can drastically modify the health policy chosen.

If the intake-risk function is linear (curve A in the diagram), everyone with an intake above  $I_A$  is above the acceptable level of risk, and in the example this constitutes the majority



of the population. If however the asymptotic function, curve B, applies only the small fraction of the population consuming more than  $I_B$  is at risk.

Clearly the difference in size of these risk groups means there will be great differences in the effectiveness of nutrition policies aimed at the whole population. Moreover, since measures to reduce disease will, in general, impose on those not at risk, the political implications, and the long term acceptability, of such policies will also differ markedly.

If nutrition theories are to be translated into nutrition policy, the quantification of the currently accepted relationships between risk factors and disease is more important than simply trying to increase our certainty about the strength of that relationship.

A change in the direction of nutritional research from causality to risk is a major one, and many scientists may view it with suspicion. But since in the foreseeable future nutritional knowledge will be chiefly applied through social policy, it is a change that must occur.

One further change seems probable. At present where nutrition policies exist at all, they have been evolved by senior administrators in closed counsel with leaders of the health professions. Such cabals will not survive for long. Misgiving about the role of scientists in government have grown to a general lack of confidence about "establishment science" as the debates over the Dangerous Pathogens Advisory Group or Windscale have made clear. It seems likely that general public concern about the role of nutrition in disease, will soon be matched by public demands to be involved in framing the policies necessary for change.

Such a change will underscore the irrelevance of the nutritionists' professional standards of certainty. Indeed they will strip away much of the veneer of professionalism that the subject has acquired. But once nutritionists recognise the essentially political nature of decisions about food policy, and the necessarily tentative nature of the theories that underpin them, we believe that they will welcome the involvement of consumer organisations and other pressure groups in the consultative process. It is reasonable that they should. At present while some scientific advisers strive after absolute proof in their decision making, the food industry employs others as spokesmen in the scientific debate, exerts massive influence on the direction of research by the funds it deploys, and by advertising pressures continues to manipulate food habits in ways that are widely suspected as having led to increased disease. A change in the way we tackle these problems is long overdue. □



# correspondence

## Select Committee's future

SIR,—In its recent First Report (17 July, 1978) the Select Committee on Procedure proposed some important and far-reaching changes in the procedure of the UK House of Commons. These include the establishment of a separate select committee for each of the departments of government, or groups of departments.

Our concern in this letter is with only one of the proposals, namely the dissolution of the Select Committee on Science and Technology whose work, it is said (paragraph 5.40), "should in future be carried out by the departmentally-related committees . . ." This proposal is supported in the report by an argument to the effect that the proposed new committees "would in no way be inhibited from taking evidence from any government department or other body relevant to the studies which they undertake."

Nevertheless, it is the view of the Executive Committee of this council that such a decision would be a retrograde development. We strongly support the opinion expressed to the Select Committee on Procedure by Mr Arthur Palmer MP, the Chairman of the Select Committee on Science and Technology, as indicated in paragraph 5.40 of the First Report. Departmental committees, said Mr Palmer, could not "adequately meet the need for parliamentary scrutiny of scientific and technological developments" since "inter-departmental subjects, however important they might be, would be accorded a lower priority".

The Committee on Procedure's argument depends on the generality of the policy of spreading science research and development amongst the departments of government. From many points of view there are good reasons for continuing this policy, where "after brief experiments in the 1960s with co-ordinating ministries, governments of all complexions have decided to leave scientific and technical research in the hands of individual ministries with only vestigial co-ordinating functions remaining at the centre" (paragraph 5.19). It does not follow from this, however, that the *scrutiny* of these activities should be compartmentalised to the same degree. Indeed, it is precisely because the central coordinating functions for scientific and technical research are vestigial that the work of the Select Committee on Science and Technology is so valuable.

Presumably, within the proposed system, there would be parliamentary committees for each of the Departments of Education and Science, Energy, Environment, and Industry, and perhaps other departments as well, all dealing with particular aspects of science and technology. Such committees would, undoubtedly, serve a valuable purpose in scrutinising the day-to-day, ongoing, short-term, research that is central to the work of each department. But no amount of "liaison and cooperation" between such committees "to allow joint inquiries and consultation, where committees share an interest" (paragraph 5.20), could deal satisfactorily with the broader, long-term, issues of

science and technology.

This can be seen at once from the issues that have already been taken up by the Select Committee on Science and Technology. These were necessarily concerned with scientific and technological questions drawn from a whole range of departments such as energy, environment, industry, health and social security, etc. Current issues of great political, social and economic significance, such as the potential benefits and hazards of genetic manipulation or the future effects of electronic microprocessors, obviously overflow all the boundaries appropriate for the administration of science and research within the government machine. By its terms of reference, which encourage it to scrutinise positively the activities of the executive on such issues right across the board, the Select Committee on Science and Technology fulfils an indispensable role on behalf of the lay public which its members are elected to represent. From the experience of our council and the detailed studies that we have made of policies and procedures relating to the social and political context of science and technology, it is clear that this is a role that should be considerably strengthened, rather than weakened, as proposed. As an institution endowed with all the constitutional authority of Parliament, capable of mediating effectively between the various domains of scientific expertise, governmental administration, and the concerns of the general public, the Select Committee on Science and Technology is potentially one of the most valuable social instruments available to us in times of rapid and bewildering technical change.

In the past the Select Committee on Science and Technology has not always been able to achieve this full potential. From our own close contact with members of that committee, and from our observation of its work, this is not due to any fundamental defect in its terms of reference, but rather to the very limited permanent staff resources, and restrictions on the appointment of specialist advisors, under which the committee has laboured. These deficiencies are recognised in the First Report from the Select Committee on Procedure, which recommends much improved arrangements in this respect. In our view, it is ironical that these resources should now be made available to new select committees which are to be much more closely linked to particular departments where technical staff resources abound, whilst proposing to terminate a committee which has been weakened by lack of such resources.

In our view, the proposed new system would perpetuate, in a most unsatisfactory manner, the split between science and technology which occurred when the DES was first created. Furthermore, there are many areas within technology itself which cannot be neatly divided into the interests of Departments of Energy, of the Environment, of Industry, etc. This fragmentation is not merely inconsistent with the realities of modern scientific research and technological innovation. It also takes a narrowly instrumental view of the contributions of various expert

disciplines to the issues raised by technical change. It is not so much, as the report suggests (paragraph 5.42), "that scientific and technical matters in widely varying fields involve a common approach which can best be handled by common methods"; it is rather that many current issues and policies cannot be adequately scrutinised except from a point of view that transcends and integrates the separate departments of government. The various proposed departmental committees, however loose their terms of reference and however well "coordinated" they might be over particular issues, could not adopt a sufficiently comprehensive viewpoint where social, environmental, economic, health and safety and industrial factors would be related to narrower technical or administrative matters.

We hope, therefore that Parliament will consider very carefully before dissolving the Select Committee on Science and Technology. Whether or not departmental committees are created, we believe that the Select Committee on Science and Technology should be retained. Its broad remit it seems to us, is just what is needed for the purpose of taking an overview of the various areas where pure science interacts with all the separate branches of technology, and where these branches interact with each other and where they may also have far-reaching social consequences.

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## Scientist-entrepreneurs

SIR,—One aspect was not mentioned in the article by Dickson (5 April, page 494) concerning the commercial payoffs of recombinant DNA research. It is clear from the article that one of the 'fall-outs' of recombinant DNA research is the emergence of a group of scientist-entrepreneurs. Practically all the research which has led to the discovery and techniques of recombinant DNA was funded by public monies. It is obvious from the article that the recipients of these grants have jumped in with both feet in aiding to set up commercial companies to capitalise on the possible future profits resulting from the large-scale exploitation of recombinant DNA techniques. One wonders who will gain the monetary profits—only the stockholders of the companies or the scientist-entrepreneurs as well. One also wonders at the disinterestedness of the scientists, who clamoured for no restrictions on the research, giving as a reason the possible great public health benefits which might result. It seems clear now that if the benefits materialise they will accrue not only to the public, but also to the companies and the scientists. It seems to me that there is an ethical issue in all this, for while in our capitalistic society companies are supposed to make money, in our scientific society scientists are supposed to search for 'the truth', for the benefit of all and are not supposed to make money from their research, particularly if it was funded by public monies.

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# news and views

## Unsuspected relatives of the ovalbumin gene

from Norman Carey

THE power of cloning techniques in the analysis of the eukaryotic genome is once again dramatically demonstrated by a paper published in this issue of *Nature* (page 125). Using the new methodology, genes apparently related to, and lying close to, the ovalbumin gene have been discovered. It seems unlikely that any other procedures would have uncovered their existence. This paper, whilst revealing yet further complexities in the organisation of the eukaryotic genome, could also lead to studies in which the control of gene expression by steroid hormones can be elucidated.

The abundant capability of the avian oviduct for protein synthesis which is under the control of steroid hormones, has lured a number of major groups to study this tissue in the hope of discovering the basis of at least one mechanism of control of protein synthesis. The early phases of this work (reviewed for example by Palmiter *Cell* **4**, 189; 1975 and O'Malley & Means *Science* **182**, 610; 1974) established that oestradiol, with or without other hormones, stimulates the growth of the tissue and the synthesis of the four major egg white proteins, ovalbumin, conalbumin, ovomucoid and lysozyme, which together can account for over 80% of the protein synthetic capacity of the cell (Palacios *et al. J. biol. Chem.* **247**, 2316; 1972). It has also been shown that the presence of the hormones promotes the accumulation of the messengers for these proteins, and if the hormones are withdrawn, the messenger content of the tissue declines to very low levels. This, and similar observations, has led to the proposition that oestrogens induce the synthesis of the proteins by a process which is the eukaryotic equivalent of the Jacob-Monod hypothesis of operon induction, a contention which has proved very difficult to support. The three effective steroids, oestradiol, progesterone and testosterone, affect the synthesis of the

major protein components differentially, in such a way that the induction process seems likely to be very complex. The experiments designed to test the proposal have been open to more than one interpretation and, in addition, other effects of steroids have been observed, such as the ability to stabilise mRNA (Palmiter & Carey *Proc. natn. Acad. Sci. U.S.A.* **71**, 2357; 1974).

This phase of primarily kinetic studies drew to a close with the advent of gene cloning. This provided a way of analysing the structure of the genes in the genome, and thus identifying the control regions which are postulated to be adjacent to the genes and are presumed to associate with polymerases and controlling proteins. The cloning of the ovalbumin gene has been dominated by the work of two groups, those of O'Malley in Texas and Chambon in Strasbourg. The first step for both was to clone the structural gene obtained from mRNA. In O'Malley's laboratory, this led first to the elucidation of the sequence of the mRNA, in collaboration with George Brownlee at the MRC Laboratory of Molecular Biology, Cambridge, and was obtained just at the time that the Fothergills in Aberdeen were putting the finishing touches to the polypeptide sequence (McReynolds *et al. Nature* **273**, 723; 1978). Chambon's group concentrated its attention on the genomic structure, and they were amongst the first to observe the presence of non-translated intervening sequences (introns) in the genes coding for polypeptides in eukaryotes (Breathnach *et al. Nature* **270**, 314; 1977; Deol *et al. Nucl. Acids Res.* **4**, 3701; 1977). The precise details of these intron/exon arrangements then rapidly became clear from the work of both Chambon's and O'Malley's groups (see for example, Garapin *et al. Cell* **14**, 629; 1978; Dugaiczky *et al. Nature* **274**, 328; 1978; Gannon *et al. Nature* **278**, 428; 1979). The sequence which appears in the cytoplasm as messenger is interrupted in no less than seven places in the genome by DNA sequences varying from about 0.3 to 1.4 kilobase pairs. All but one of these

interruptions are found in the 5' half of the molecule which, because of the long 3' untranslated region of ovalbumin mRNA, puts them all in the coding region. The remaining intron is in the 5' untranslated region. A great deal of information has been obtained about features of the system which are shared with other genes, such as the presence of RNA precursors of messenger which are tailored to the final structure (Roop *et al. Cell* **15**, 671; 1978; Chambon *et al. Proc. 11th Miami Winter Symp.* in the press) and the nature of the sites in the RNA which are recognised by the processing enzyme (Catterall *et al. Nature* **275**, 510; 1978; Breathnach *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 4853; 1978). There are, however, other features which, while so far peculiar to the oviduct genes, must be taken into account when considering the question of eukaryotic genome organisation.

The function of introns in eukaryotic structural genes has been the subject of much speculation, and perhaps the most acceptable suggestion is that they flank regions of the gene coding for domains in the polypeptide to which portions of the total function of the protein can be ascribed. It is postulated that the genomic DNA regions coding for these domains have been brought together during evolution by a process involving the introns (Sakano *et al. Nature* **277**, 627; 1979; see *News and Views* **277**, 598; 1979). However, it is hard to see how this applies to ovalbumin. No specific functions have been ascribed to this protein. It may have important nutritional, hydrodynamic or other similar properties in relation to maintaining the developing embryo, but no function has been described or postulated which would require the cooperation of seven separate functional domains in the polypeptide. However, the function of the first intron of the ovalbumin gene must certainly be different since it lies in the 5' untranslated region of the mRNA and so has no relationship with any functional domains of the polypeptide. (Breathnach *et al. Proc. natn. Acad.*

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*Sci. U.S.A.* **75**, 4853; 1978; Royal *et al.* this issue of *Nature*, page 125). Similarly, in the ovomucoid gene, a large number of exons has been found in the genomic DNA coding for a small protein which seems unlikely to have a complicated function requiring the assembly of a series of polypeptide domains (Catterall *et al.* *Nature* **278**, 323; 1979). Nevertheless, the polypeptide domain hypothesis remains an attractive one and it is possible that domain reassortment has occurred during the evolution of the oviduct proteins, but for reasons unrelated to the function of the polypeptide.

The latest piece of work in this fascinating series is published in this issue of *Nature* and is another tour de force from the powerful teams of Chambon and Kourilsky. Clearly, if there is any feature of the eukaryotic genome which resembles an operon in relation to hormonal induction, features in the DNA regions surrounding the ovalbumin gene would be expected to reveal it. Chambon and Kourilsky have therefore begun to 'walk along' the genome proceeding outwards from ovalbumin. To do so, they have used a new generation of recombinant DNA vectors called 'cosmids' which combine some properties of plasmids with the high efficiency of bacteriophage *in vitro* DNA packaging procedures, giving a very efficient selection of recombinants containing large sections of foreign DNA. Various partial digests of the chicken genome were cloned by Chambon and Kourilsky and were selected by hybridisation to a probe which covers the 5' region of the genomic ovalbumin gene, both introns and exons. Two clones are described in some detail. One, pAR1, starts within the third ovalbumin intron and extends for a total of 17 kb pairs in the 3' direction, about 12.5 kb pairs being outside the ovalbumin gene. The second, pAR2, is a 30 kb pair fragment which extends in a 5' direction from within the ovalbumin gene, and includes its first four exons. The two fragments thus cover a total of about 46 kb pairs, and overlap within the ovalbumin gene, which occupies about 7.7 kb pairs.

Certain peculiarities in the hybridisation data led the group to question whether the regions which they had cloned contained any other genes which are expressed in the oviduct. This was assessed by hybridising the total poly(A)-containing RNA from oviducts with the clones, and examining the hybrids in the electron microscope. No other genes were found on the 3' side of the ovalbumin gene; that is in the pAR1 clone, but two were found on the 5' side in pAR2. They are named X and Y, gene X being the most distal from ovalbumin, and its 5'

region seems not to be present in the clone but the RNA which hybridises to it is 2,400 nucleotides long. Gene Y RNA is about 2,000 nucleotides long, near to the size of ovalbumin mRNA, and the gene, like ovalbumin, has seven introns. The first intron of gene Y is close to the 5' end, in both X and Y all the introns are in the 5' half of the gene, and the exons of X, Y and ovalbumin are of similar size. This similarity of intron-exon pattern is not all. Parts of these genes cross hybridise with each other, and both with parts of the ovalbumin gene, which accounted for the hybridisation peculiarities which set the work on their trail in the first place. The conclusion is that there are sequence homologies in parts, but not all, of the expressed regions of all three genes.

There is also good evidence to show that the expression of all three genes is under hormonal control. The messengers are present in oviducts from birds treated with oestrogen but disappear when the hormone is withdrawn. The quantitation of this expression awaits further work, but the indications are that X and Y are only expressed to a small extent compared with ovalbumin. Although the data are not presented, the authors state that neither X nor Y codes for any of the other abundant oviduct proteins conalbumin, ovomucoid or lysozyme, but which proteins they do code for, and whether the messengers are in fact translated in the oviduct, remains unknown for the present. There is a great similarity between the organisation of the ovalbumin-related genes and that of the human  $\beta$  globin-related genes (see Little *et al.* *Nature* **278**, 227; 1979), where four genes (two  $\gamma$  genes,  $\delta$  and  $\beta$ ) of similar intron pattern are found close together on the genome. In the case of both gene sets, the 5' to 3' orientation of the genes is the same, and they are transcribed in the same direction. A further interesting feature is that the genes of each set are not expressed to the same extent under the same conditions, ovalbumin predominating over X and Y in the oestrogen-treated oviduct, and the  $\beta$  globin expression varying with the life cycle or disease state. We may now expect that information on the relatedness of the exons of the ovalbumin cluster will be useful in suggesting how these genes were assembled during evolution. The hybridisation data indicate that some exon regions of ovalbumin, X and Y, are closely related whereas others are more distantly related. The fine details of this relationship, perhaps including comparisons with other avian species, should indicate whether the genes have been assembled in parallel during evolution from a mixture of closely

related and unrelated regions, as the hybridisation data seem to suggest. In that case, the similarity in size of the exons may turn out to be coincidental. Alternatively, the cluster may have arisen by duplication of an ancestral gene, thus fixing the intron-exon pattern from the outset, but leaving the need to find an alternative explanation for the differing relatedness of the exons.

As for hormonal control, it has to be admitted that we are as yet no further ahead in our understanding. However, the close proximity to the genome of genes which are expressed to different extents under the influence of one hormone poses the question of whether transcription of the genes is initiated separately and with differing efficiency commensurate with the messenger abundance, or whether they are transcribed to the same extent by the same polymerase, operon fashion, with subsequent processing and stabilisation accounting for the differing levels in the cell. In other words the time may now be approaching when workers in the field can return to the study of the kinetics of the induction process with some real hope of testing the hypotheses. □

## Histocompatibility antigens and cytoskeletal elements

from Franklin H. Portugal

MAJOR histocompatibility antigens designated as H-2 in mice and HLA in man are cell-surface glycoproteins whose movement in the plasma membrane appears to be associated with the movement of intracellular cytoskeletal elements. These antigens are involved in such immune reactions as allograft rejection and recognition of viral and tumour antigens by syngeneic cytotoxic thymus-derived (T) lymphocytes. Initially, it was difficult to isolate even a few micrograms of pure HLA antigen from human splenic lymphocytes. Observations that the surface of cultured human lymphoblastoid cells contains a greatly enhanced antigen concentration simplified this problem and resulted in the isolation of milligram amounts of material. In 1975, Peterson *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **72**, 1612; 1975) proposed that the large chain for H-2 and HLA antigens, already known to carry the alloantigenic determinants, has both

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chemical and physicochemical properties similar to those of immunoglobulin. These antigens also contain a smaller chain which consists of  $\beta_2$ -microglobulin, is invariant, and shows homology to various IgG heavy and light chain homology regions. Because major histocompatibility antigens are found on the surface of virtually all normal, nucleated cells in man and mice, probably all mammalian cells have an immunoglobulin-like molecule on their surface. By solubilising H-2 antigen with either a proteolytic enzyme (papain) or detergent, Henning *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **73**, 118; 1976) showed that the amino-terminal end of the heavy chain extends away from the cell surface whereas the carboxyl-terminal end is associated with the cell membrane. Further characterisation indicated that H-2 antigen consists of a light-chain  $\beta_2$ -microglobulin (12,000 daltons) and a heavy polypeptide chain (46,000 daltons) that carries both distinctive antigenic determinants as well as a carbohydrate prosthetic group (Edelman *Science* **192**, 218; 1976). Similar studies with HLA-A antigen showed that it contained a light chain of  $\beta_2$ -microglobulin (12,000 daltons) and a heavy polypeptide chain (44,000 daltons) containing both antigenic specificity and carbohydrate (Springer & Strominger *Proc. natn. Acad. Sci. U.S.A.* **73**, 2481; 1976). Springer and Strominger proposed that a hydrophobic region of the heavy chain is inserted into the cell membrane so that the amino-terminal portion of the chain extends outside the cell and the carboxyl-terminal portion extends into the cell interior. By using the lactoperoxidase-catalysed iodination of the inner surface of lymphocyte plasma membrane, Walsh and Crumpton labelled HLA and showed therefore that this antigen is a transmembrane protein (*Nature* **269**, 307; 1977).

How the structure of these antigens and their insertion in the cell membrane may affect immune reactions can be studied with liposomes. Littman *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **76**, 902; 1979) have prepared unilamellar phosphatidylcholine liposomes containing H-2 antigen. In these liposomes, H-2 antigen was found to be integrally inserted in the membrane, oriented with the amino-terminal end of the chain facing away from the cell surface, and antigenically active. Such antigen-containing particles can also be used in assays with T lymphocytes.

Antigen movement over the cell surface is detectable by patching and capping phenomena. Bourguignon and Singer used a double fluorescence technique for staining both surface-bound H-2 antigen on mouse splenic T lymphocytes and for staining intracellular mechanochemical proteins such as actin or myosin (*Proc. natn. Acad. Sci.*

*U.S.A.* **74**, 5031; 1977). When the surface-bound receptors collected in patches in response to an external multivalent ligand, the membrane-associated actin or myosin within the cell also accumulated in patches directly under those of the receptor. This suggests that H-2 glycoprotein becomes linked to the intracellular contractile protein, possibly through an integral class of proteins that are located in the plasma membrane of eukaryotic cells. Koch and Smith confirmed that there is an association between H-2 antigen and actin (*Nature* **273**, 274; 1978), although the question of whether the association is a direct or indirect one could not be resolved.

Pober *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **75**, 6002; 1978) have recently indicated how such an association between the intracellular domain of a transmembranous protein and the cytoskeletal protein may be regulated. Incubation of either transformed lymphoblastoid cells or peripheral blood lymphocytes with  $^{32}\text{P}_i$  resulted in the phosphorylation of HLA-A and HLA-B antigens. The site of phosphorylation

is a serine residue(s) located in the hydrophilic, carboxyl-terminal region of the heavy chain. Incubation of isolated membranes from transformed lymphoblastoid cells with  $[\gamma^{32}\text{P}]\text{ATP}$  also produced phosphorylation of HLA antigens. Because interaction with actin is known to occur with many proteins that are phosphorylated, the phosphorylation of HLA antigens may be the basis for regulating association with cytoskeletal elements.

Many questions remain to be answered in future experiments. One concerns what factors regulate the phosphorylation of these membrane proteins. The question of which serine residue(s) of HLA-A and HLA-B antigens are phosphorylated and the number of phosphate groups incorporated into each heavy chain requires further resolution. Presumably, HLA antigen binds to actin as does H-2 antigen but this also remains to be demonstrated. It is still unclear whether microfilaments or microtubules or both are the cytoskeletal elements involved in the association with transmembranous proteins.  $\square$

## Laser spectroscopy pins down Rydberg constant

from John E. M. Goldsmith

The availability of tunable laser radiation and associated nonlinear spectroscopic methods has revolutionised many areas of spectroscopy (for a review, see *High-Resolution Laser Spectroscopy* Ed. Shimoda, Springer-Verlag, Berlin, 1976). One notable example is the elimination of Doppler broadening in the observation of the Balmer-alpha line ( $n=2 \rightarrow n=3$ ,  $\lambda=656 \text{ nm}$ ) of atomic hydrogen with the technique of saturated absorption spectroscopy (Hänsch, Shahin & Schawlow *Nature* **235**, 63; 1972). The Doppler effect causes atoms moving with different speeds to appear to have different resonance frequencies, just as the apparent pitch of a train whistle changes as the train moves past the observer. Since the atoms in a gas have a wide range of velocities, and all of the atoms are observed using classical (linear) spectroscopic techniques, the net effect of the Doppler shift is to cause a broadening of the spectral line. With saturated absorption spectroscopy, only atoms that are not moving (or at least have no component of velocity along the laser beams) are observed, thus eliminating Doppler broadening. The increase in resolution obtained in this

manner makes it possible to observe the individual fine structure components of the Balmer-alpha transition.

The ability to resolve this fine structure has great importance for measurements of the Rydberg constant. The Rydberg, which is defined as an explicit combination of other fundamental constants  $R = \mu_0^2 m_e e^4 c^3 / 8h^3$ , is a cornerstone in the evaluation of the fundamental physical constants. The physical meaning of the Rydberg is most clearly seen in the simple Bohr theory, in which the energy levels of the hydrogen atom are given by the expression  $E_n = -hcR/n^2$ ,  $n=1, 2, 3, \dots$ ; the principal quantum number  $n$  labels the energy levels of the atom. Thus the value of the Rydberg constant corresponds to the energy required to ionise the ground state hydrogen atom.

Until 1974, the Rydberg constant was determined by measuring the wavelength of emission lines from cooled hydrogen, deuterium, tritium, or ionised helium discharges (see *Series, Contemp. Phys.* **14**, 49; 1974, for a review of these measurements). The fine structure components of the lines were not resolved because of Doppler broadening, so it was necessary to calculate the spectral lineshape produced by the blend of components. This caused an uncertainty in pin-

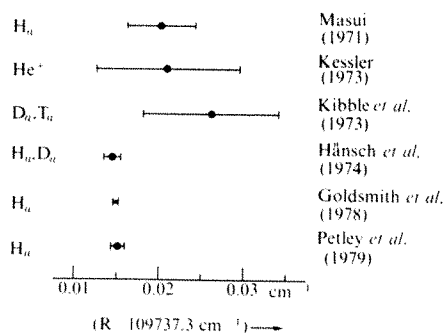
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pointing the 'centre' of the emission line, and thus an uncertainty in the value of the Rydberg constant.

Resolution of the fine structure of the Balmer-alpha line with Doppler-free laser spectroscopy has made possible nearly two orders of magnitude improvement in the precision of the Rydberg constant, making it one of the (if not the) most precisely measured of all fundamental physical constants. The first Doppler-free measurement used saturated absorption spectroscopy with a pulsed dye laser to observe the hydrogen and deuterium  $2P_{3/2}-3D_{5/2}$  transitions. A d.c. discharge tube similar to Wood's original design was used to dissociate the hydrogen molecules and excite some of them to the  $n=2$  level. The Rydberg constant was determined from a measurement of the wavelength of this transition, using an iodine stabilised helium-neon laser ( $\lambda=633$  nm) as a wavelength standard. The value so obtained,  $R=109737.3143(10)$   $\text{cm}^{-1}$  (Hänsch *et al.* *Phys. Rev. Lett.* **32**, 1336; 1974), was an order of magnitude more precise than previous Doppler-broadened measurements.

Further improvement in the Rydberg constant has been aided by the development of narrowband continuous wave (cw) dye lasers, and suitable dyes for cw operation at the Balmer-alpha wavelength. Using a variation of saturated absorption spectroscopy (known as polarisation spectroscopy) with a cw dye laser, and replacing the Wood's tube with a mild helium-hydrogen discharge, it became practical to study the hydrogen  $2S_{1/2}-3P_{1/2}$  transition, which is weaker than the  $2P_{3/2}-3D_{5/2}$  transition, but also more than three times narrower. The wavelength measurement of this transition was facilitated by first determining the wavelength of an iodine reference line which by good fortune has a wavelength almost identical to that of the  $2S_{1/2}-3P_{1/2}$  transition. A measurement of this small wavelength difference then yielded the value  $R=109737.31476(32)$   $\text{cm}^{-1}$  (Goldsmith, Weber & Hänsch *Phys. Rev. Lett.* **41**, 1525; 1978), with a precision three times higher than obtained previously. A more precise measurement of the wavelength of this iodine reference line could immediately yield at least another twofold improvement in the precision of the Rydberg constant.

Another recent Rydberg measurement returned to the use of saturated absorption spectroscopy in a Wood's discharge tube, but with a narrowband cw dye laser. The wavelength of three hydrogen Balmer-alpha fine structure components were measured ( $2P_{3/2}-3D_{5/2}$ ,  $2S_{1/2}-3P_{3/2}$  and  $2P_{1/2}-3D_{3/2}$ ), with the iodine-stabilised helium-neon laser again used as a wavelength



standard. The weighted mean of these values yielded the result  $R=109737.31513(85)$   $\text{cm}^{-1}$  (Petley and Morris, this issue of *Nature* page 141).

The values and precision of Rydberg measurements from the past 10 years are summarised in the figure. The Doppler-free measurements clearly are much more precise than the earlier Doppler broadened measurements. It is more significant, however, that these three independent measurements agree very well with each other, but are only marginally consistent with the earlier determinations. The possibility of such a systematic error indicates the need for independent measurements of values such as the Rydberg constant. The excellent agreement of the three Doppler free determinations, performed in two laboratories on several Balmer-alpha fine structure components using varied techniques, is a strong indication that such a systematic error has been avoided.

Measurement of the Rydberg constant at the current level of precision is already hindered by uncertainties inherent in the  $^{86}\text{Kr}$  primary wavelength standard, which limit its useful precision to a few parts in  $10^6$ . This is an artificial difficulty, however, and can be avoided by using lasers stabilised to molecular transitions, such as the iodine-stabilised helium-neon laser, as interim wavelength standards. Direct frequency measurements have recently been extended into the infrared region of the spectrum, and frequency measurements in the visible may not be too far off. For measurements with a precision greater than 1 part in  $10^6$  it will probably prove necessary to work with an atomic hydrogen beam, so small energy level shifts caused by conditions in the discharge can be eliminated. The ultimate Rydberg measurement could come from a frequency measurement of a narrowband cw ultraviolet laser source ( $\lambda=243$  nm,  $\nu=10^{15}$  Hz) used to excite the  $1S-2S$  transition in hydrogen using Doppler-free two-photon spectroscopy (Hänsch *et al.* *Phys. Rev. Lett.* **34**, 307; 1975). The natural linewidth of this transition is about 1 Hz, so one

can conceive of a measurement of its frequency with a precision better than 1 part in  $10^{15}$ . Using modern atomic theory, it is necessary to apply several small corrections to the hydrogen energy levels calculated with Bohr's theory. With such a frequency measurement, the precision of the Rydberg constant would only be limited by the precision with which these small corrections can be calculated. □

## Ionic landmarks along the mitogenic route

from K. S. Koch and H. L. Leffert

Two ionic fluxes, akin to those initiating and transducing nerve impulses, may be essential regulators of animal cell proliferation. This idea emerged at a recent conference\* where many findings pointed to increased  $\text{Na}^+$  influx being an early, perhaps the initial, mitogenic signal, and to subsequent  $\text{Ca}^{2+}$  ion movement coupled to cyclic AMP formation being necessary for DNA replication.

Experiments using amiloride (a specific  $\text{Na}^+$  influx inhibitor) and  $^{22}\text{Na}^+$  showed that 'burst-like' influxes are needed to initiate DNA synthesis in sea urchin eggs after fertilisation (D. Epel, Stanford University), in rat hepatocytes *in vitro* after exposure to peptide growth factors including EGF (S. Cohen's epidermal growth factor) (K. S. Koch & H. Leffert, The Salk Institute), and in cultured mouse 'fibroblasts' after exposure to serum factors including vasopressin (E. Rozengurt, Imperial Cancer Research Fund, London). Similar fluxes appeared operative during phytohaemagglutinin-induced human lymphopoiesis (G. Kaplan, University of Ottawa) and veratridine-stimulated mitogenesis of cultured chick central nervous system neurones (C. D. Cone, Hampton Virginia VA Hospital).

Possible mechanisms by which growth factors stimulate  $\text{Na}^+$  influx were considered. G. Eisenman (University of California, Los Angeles) discussed current views of cation-selective channel functioning based on gramicidin models and proposed that certain mitogens directly form the  $\text{Na}^+$  channel—an idea awaiting experimental test. Indirect mitogenic actions on channel 'gating' mechanisms result-

\*Growth Regulation by Ionic Fluxes' was held on 13-17 March 1979 at the Kroc Foundation, Santa Ynez, California, and the proceedings will be published as a number of the *Annals of the New York Academy of Sciences*.

ing from altered surface potentials (C. Bergman, University of Paris); upon lateral diffusion of membrane proteins resulting from altered membrane potentials (M. Edidin, Johns Hopkins University) measured with lipophilic cations like triphenylphosphonium<sup>+</sup> (R. Kaback, Hoffman-LaRoche); and on cell-cell interactions resulting from altered electrical coupling (W. Loewenstein, University of Miami) were also discussed.

How might increased Na<sup>+</sup> influx stimulate proliferation? One attractive mechanism, generally consistent with the experimental results described, may be activation by intracellular Na<sup>+</sup> of the membrane Na<sup>+</sup>/K<sup>+</sup> ATPase 'pump'. Such changes are expected to alter Na<sup>+</sup> gradients across the surface membrane. The coupling of solute transport to these gradients through a membrane 'carrier'—presumably to stimulate biosynthetic pathways—was reviewed in detail by R. Crane (Rutgers University). He showed evidence for amiloride-sensitive Na<sup>+</sup> gradient-dependent glucose uptake into rabbit intestinal vesicles as well as reconstitution of this system with a purified protein, obtained from brush-border membrane fractions, inserted into liposomes. The effects of growth factors in Crane's vesicle systems are unknown. Another possible consequence of increased Na<sup>+</sup> influx studied in fertilised eggs—a Na<sup>+</sup>/proton exchange elevating intracellular pH—generated a lively debate between its proponent (Epel) and its antagonist (L. F. Jaffe, Purdue University). Epel speculated that small pH changes cause widespread alterations involving critical enzyme cascades and macromolecular polymerisations. Jaffe then described egg experiments where injected buffers that blocked alkalisation failed to block growth. F. M. Harold (National Jewish Hospital, Denver) added that disruption of proton gradients with carbonylcyanide m-chlorophenylhydrazone in bacterial systems failed to impair growth seriously. But the issue was not resolved, and the role of Mitchell's proton gradients as regulators of animal cell proliferation remains to be explored. Further consequences of increased Na<sup>+</sup> influxes were suggested by J. Piatigorsky (National Institutes of Health) and G. Koch (University of Hamburg) whose results with ocular lens and viral protein regulatory systems, respectively, raised the possibility of Na<sup>+</sup> and K<sup>+</sup> selective translational and/or post-translational controls. These inquiries may soon yield definitive conclusions, especially

if a factor isolated by G. Koch, a dialysable methionine-containing translational inhibitor released from ribosomes by high Na<sup>+</sup>, is purified and if Piatigorsky's applications of recombinant DNA technology are successful.

A fourth likely consequence of increased Na<sup>+</sup> influx was elegantly demonstrated by Jaffe who showed by the use of aequorin, a Ca<sup>2+</sup>-sensitive photoluminescent protein, the flow of a Ca<sup>2+</sup> 'wave' across the cytoplasm of a fertilised Medaka egg. If this 'explosive' wave was blocked by intracellular injections of Ca<sup>2+</sup>-chelators, DNA synthesis did not begin. Jaffe theorised that Ca<sup>2+</sup> 'currents', detected extracellularly by a 'vibrating' probe developed earlier in his laboratory, cause self-electrophoresis of membrane proteins for which evidence was presented (K. Robinson, National Jewish Hospital). Whether such Ca<sup>2+</sup> currents flow across somatic cells stimulated to divide is not yet known. However, a clear late G<sub>i</sub>-requirement for surface Ca<sup>2+</sup> movement was demonstrated—for a broad spectrum of normal cells *in vivo* and *in vitro*—without which deoxyribonucleotide, and consequently DNA, synthesis cannot occur (A. Boynton, NRC, Ottawa). These events are mediated by cyclic AMP and it was postulated that cellular ribonucleotide reductase function requires a phosphoprotein regulator whose formation depends on cyclic AMP-activated protein kinase.

Properties of nervous system and limb regeneration were reviewed by R. Moore (University of California, San Diego) and by Jaffe, respectively. An attempt was made to consider these processes with regard to the ionic events described above. Two provocative links were provided, first from Jaffe's evidence that chronically applied cathodal Na<sup>+</sup> currents stimulate partial limb regeneration in frogs and second, from F. Westall's (The Salk Institute) observations that proteolysis converts myelin basic protein into a mitogenic peptide whose sequence (Thr-Pro-Pro-Ser-Gln-Gly-Lys) is contained within the brain fibroblast growth factor (FGF) isolated by D. Gospodarowicz (University of California, San Francisco). Such findings immediately provide one explanation for nerve requirements in limb regeneration. Moreover, although Gospodarowicz showed that myoblasts, chondrocytes and fibroblasts proliferate in response to FGF, he argued that blastemal cells, from which regenerated tissue arises, may be this mitogen's natural target. Thus, Jaffe's Na<sup>+</sup> 'currents' in regenerating stumps might actually be driven by FGF-like mitogens released from crushed myelinated nerves.

The existence of two regulatory ionic

'landmarks' has interesting implications for understanding growth regulation in general. In animal cell systems, it helps to explain how sets of growth factors may act synergistically for it predicts that certain substances activate Na<sup>+</sup> influx while others act predominantly upon Ca<sup>2+</sup>-cyclic AMP coupling. Reason to believe that two-signal models are correct was presented for hepatocytes (K. S. Koch & Leffert; Boynton) and has been reported elsewhere for BALB/c 3T3 cells (see for example Stiles *et al.* *Proc. natn. Acad. Sci. U.S.A.* **76**, 1279; 1979). Furthermore, interference with the Na<sup>+</sup> flux system perturbs transitions from resting to growing states differently from interference with the Ca<sup>2+</sup>-cyclic AMP couple. The former seems to determine both the lag-duration (or 'onset' time) and the total numbers of responding cells (K. S. Koch & Leffert; Rozengurt) whereas the latter mechanism seems to regulate the rate of entry into S-phase (Boynton). These findings must be considered by any theory of growth regulation; single-event models are insufficient to account for such observations. Lastly, it seems that control of both events is defective in tumour cells (Cone; Rozengurt; Boynton) but the real significance of these observations remains to be determined. □

## Studying surfaces

from M. A. Chesters

RECENT progress in understanding the interaction of molecules with solid surfaces was a major theme of the ECOS2 Conference at Cambridge\*. Although the subjects covered included adhesion, Auger microanalysis and the chemistry of polymer surfaces, about 70% of the papers were concerned with chemisorption on metal surfaces. D. Tabor (Cavendish, Cambridge) in his opening address traced the history of surface science and urged that increased attention be paid to fundamental studies of liquid/solid and solid/solid interfacial phenomena. As it was the conference provided an excellent review of progress in fundamental studies of chemisorption, surface elemental analysis and surface structural analysis.

A major motivation for studies of chemisorption is the need to understand heterogeneous catalysis on a molecular level and two approaches to the problem of linking research on idealised systems to the behaviour of

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\*The Second European Conference on Surface Science was held at the Cavendish Laboratory and Churchill College, Cambridge, England, on 26–29 March 1979.



real catalysts were described by G. A. Somorjai (University of California, Berkeley) and R. J. Madix (Stanford University). Somorjai has pioneered work on high index (stepped) metal single crystal surfaces in an attempt to identify the types of active site involved in various catalytic processes. He described experiments carried out in an ultra-high vacuum system combined with high pressure cell, enabling metal single crystal catalysts to be characterised by low energy electron diffraction and Auger spectroscopy before and after catalysing reactions at pressures up to 200 atmospheres. Apart from this direct approach, molecular beam studies of reactive scattering from stepped surfaces revealed the importance of step and kink surface sites in breaking carbon-hydrogen and carbon-carbon bonds. Madix argued that surface intermediates important in reactions catalysed at high pressure and high temperature, may be isolated in chemisorption studies at low pressure and low temperature. He described the use of thermal desorption spectroscopy coupled with photoelectron spectroscopy to identify a surface formate intermediate in the decomposition of formic acid on Ag(110) and a surface methoxy intermediate in the decomposition of methanol on Fe(100).

In the measurement of surface reaction kinetics, molecular beam scattering remains the major technique and was reviewed by G. Ertl (München University). G. W. Rubloff (IBM, New York) described a rather more direct approach to the study of rapid surface reactions in which surface concentrations were monitored by ultraviolet photoelectron spectroscopy (UPS). The advantage of such a technique is that surface reactions which do not involve desorption of gas may be studied. Both B. I. Lunqvist (Chalmers University of Technology, Sweden) and T. B. Grimley (University of Liverpool) outlined theoretical formalisms for describing the dynamics of the interaction of atoms and molecules with solid surfaces which should pave the way for the calculation of sticking coefficients and the construction of chemisorption potential energy curves. The increased experimental and theoretical interest in studying the kinetics of reactions on well defined surfaces was particularly apparent at the conference. At the same time, there was an encouraging, continued trend away from purely 'technique-oriented' discussions, although this cannot be completely avoided in an area where new techniques abound.

The formidable problem of the elucidation of the geometrical and electronic

structure of metal-adsorbate complexes was discussed largely in the context of photoelectron spectroscopy. G. J. La Peyre (Montana State University) illustrated how the operation of the symmetry selection rule in UPS is revealed in angular resolved experiments using polarised radiation (PARUPS), thus allowing the symmetry of the ionised states to be determined. In a discussion of the requirements of a full theoretical analysis of photoemission from adsorbates, J. B. Pendry (SRC Daresbury Laboratory) remarked that if the theoretical analysis were straightforward we would learn little from the experiment! P. H. Citrin (Bell Laboratories) later reviewed the SEXAFS experiment in which the fine structure above an X-ray absorption edge, which results from diffraction of the photoelectron, may be analysed on a straightforward kinematical basis to yield accurate data on the relative positions of atoms surrounding the photoelectron emitter. Surface Extended X-ray Absorption Fine Structure is detected through modulations in the Auger yield from the absorbing atom to achieve surface sensitivity, and Citrin illustrated the power of the technique by reporting results for iodine adsorbed on silver and copper single crystal surfaces. The chief disadvantage of SEXAFS is that a synchrotron radiation source is essential, so we shall be hearing much more of the technique as storage rings dedicated as photon sources become operational.

In the past few years there has been a resurgence of electron energy loss spectroscopy (EELS) as a means of studying vibrational modes of adsorbed species. This is in large measure attributable to H. Ibach (KFA, Jülich) and he gave an account of its application to the study of hydrocarbon adsorption on nickel surfaces. The technique involves the measurement of small energy losses (40–500 meV) of electrons impinging on a solid surface which arise through excitation of vibrational modes of the surface and of adsorbed molecules. The sensitivity is about two orders of magnitude higher than for reflection absorption infrared spectroscopy. Ibach was able to show some striking differences between the reactivity of a flat Ni(111) surface and a stepped Ni[5(111)×(1 $\bar{1}$ 0)] surface towards acetylene and cyclohexane. R. F. Willis (ESTEC, The Netherlands) has carried out a detailed investigation of the W(100)/hydrogen system in which he has pioneered the use of EELS in both specular scattering and diffuse scattering modes for which different selection rules apply. He described a detailed model for a reconstructed surface in which tungsten-hydrogen-tungsten bridges are tilted

out of the surface plane. The apex angle of the bridge and the tilt angle were determined from the frequencies and angular dependences of the three hydrogenic vibrational modes. Clearly EELS can supply some of the basic structural information on adsorbates which has proved so elusive and will be a vital complement to the other electron spectroscopic techniques in the future.

There is a continuing debate on the relevance of these idealised investigations to 'real' problems in surface science (see Tompkins, *Chemistry in Britain* **15**, 194; 1979). For myself the conference was preaching to the converted. I leave others to make their own judgement. □

## Birds as agricultural pests

from John Krebs

THE male bullfinch not only looks beautiful; it can be trained to whistle German folk tunes, and given the chance it will strip half the fruit buds off a pear tree in 90 minutes. It was this last attribute which brought the bullfinch to the attention of a recent meeting on birds as agricultural pests.\* J. M. Flegg (East Malling Research Station) reviewed the damage caused by bullfinches, redpolls and other birds to fruit crops, and pointed to some common sense factors influencing the level of destruction. Orchards planted next to natural woodland are more likely to suffer bullfinch damage as the woodland provides a good home base for the birds. Alder trees planted as windbreaks draw in redpolls to feed on the cones, and when the cone supply is exhausted the birds switch to devouring fruit buds; and the habit of removing all the herbaceous weeds from orchards may increase bullfinch damage, since the bullfinch prefers weed seeds and only switches to fruit buds when seeds are not available.

Farmers have tried to alter the food preferences of bird pests for hundreds of years. Although older methods such as rubbing garlic or pigs' dung on fruit trees are now outmoded, a modern equivalent is to try and condition birds to dislike crop foods by treating the crop with a chemical which makes the birds feel sick. This kind of aversive conditioning is well known to induce

\*The Symposium on 'Understanding Agricultural Bird Problems' was organised by the British Crop Protection Council and Agricultural Science Service at Royal Holloway College, 4–5 April 1979. The proceedings are to be published (by the British Crop Protection Council) as a book edited by E. N. Wright, I. Inglis & C. J. Feare.

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'bait shyness' in rats and J. Rogers (US Fish and Wildlife Service) reported that it also works with birds. In laboratory trials, red-winged black-birds would avoid eating a foodstuff for more than 16 weeks after first encountering it mixed with the insecticide methiocarb, and this conditioned aversion was effective even when the only alternative food was highly unpalatable. Large scale field trials showed that methiocarb can reduce bird damage to vineyards by something like fivefold.

I. Inglis (Pest Infestation Control Laboratory, Worplesdon) described the efficacy of different kinds of visual scaring devices. The traditional scarecrow, and popular modern equivalents consisting of bright coloured miniature windmills are totally ineffective, and Inglis suggested that a knowledge of the behaviour of pest species is useful in designing more effective bird-scarers. For example, when a wood-pigeon is frightened and takes to the air, it reveals conspicuous white wing patches which might be seen by other pigeons as a danger signal. This led Inglis to try out the deterrent effect of pairs of pigeon wings lying on the ground, and in one study birds were largely deterred from landing in a clover field for up to 70 days by the sight of pigeon wings on the ground. Similarly, Brent geese were discouraged from landing in a field by putting out model geese in an 'alert anxiety' posture. One of the most effective deterrents of all is the sight of a man flapping his arms up and down like a giant eagle, and there is now available on the market a 7-foot high Incredible Hulk with mechanical flapping arms. All these scaring devices suffer from the problem of habituation: birds eventually learn to stop flying away from them unless occasionally reminded with a gun that there is a real danger. It may turn out in the end, as suggested in one of the discussions, that the best solution to bird scaring is the one recommended in 1668 by Gervais Markham in *Farewell to Husbandry*: "The only best and safest means to prevent this evil is to have' evensome young boys with bows and arrows to follow the seedman and harrows, making a great noise and shooting his arrows where he shall see these devourers aleight, not ceasing but chasing them from the land".

The meeting also touched on a more fundamental ecological issue raised by all techniques of scaring, deterring, or luring away bird pests: what happens to birds when they are frightened off a particular field? If they go next door, the problem is merely shifted on to someone else. One possibility, discussed by M. Owen (Wildfowl Trust, Slimbridge) is to provide refuges for birds,

## The leaning tower

from Ian Smalley

BECAUSE the Tower of Pisa was built very slowly, with many interruptions, it still stands; had it been completed during the 12th century it would probably have collapsed (Mitchell, Vivatrat & Lambe *J. Geotech. Eng. Div. Amer. Soc. Civ. Eng.* **103**, 227; 1977). Work on the foundations of the tower began in August, 1173 and the first story was completed in 1174. When the tower reached a height of three and a half storeys and a load of 9,840 metric tons in 1178 the work stopped. The work stoppage has been attributed to politics, to the heavy work load of the constructors, and to construction difficulties. Construction was not resumed until almost a century later and then the tower was completed up to the eight storey level, and to a total load of 13,728 t, during the period 1272-1278. Work then stopped again and did not resume until 1360 when the final storey was added and the whole tower completed in 1370. By the time of the final stage of construction the lean of the tower was significant and the centre line of the topmost part was changed on account of this.

Mitchell *et al.* concluded that the bearing capacity of the soils underlying the tower was never exceeded. The total settlement of the tower is due to the sum of four components: an immediate compression of the sands in a 7 m thick zone underlying the base of the tower, immediate compression of a 30 m thick clay layer underlying the sands, consolidation of the clay layer and secondary compression of the clay layer. They were unable to show, using their classical soil mechanics methods, why the tower actually leans although they suggested that this might be due to the higher compressibility of the foundation sand on the south side of the tower. Veder (*Bauingenieur* **50**, 204; 1975) had suggested earlier that the stone blocks used in the construction were stored on the south side of the tower causing an asymmetric loading.

Although the tower leans to the south now, it seems to have pointed

in various directions during its construction. From 1173 to 1178 two-thirds (in terms of weight) of the tower was constructed and it tilted towards the north-east. By the time construction resumed in 1272 this inclination had doubled. Inclination increased for a further 6 years at which time 86% of the final weight was in place. The tower then leant towards the north-west, and in 1370 when the tower was completed its inclination was towards the south, as it is today. The tilt was 1/31 in 1370 but today it is around 1/10 and, after a deceleration of movement for five and a half centuries, movements are today tending to accelerate. Much of the recent movement can be related to the removal of water from nearby wells and the lowering of the piezometric head in the deep sandy layer (Mascardi *J. Geotech. Eng. Div. Amer. Soc. Civ. Eng.* **104**, 299; 1978).

Cambefort (*J. Geotech. Eng. Div. Amer. Soc. Civ. Eng.* **104**, 156; 1978) has calculated that the tower will be stable until the year 2780, as long as the pumping from the deep aquifer does not increase beyond the 1966 rate. He has also proposed an ingenious lever mechanism to halt the tilt and fix the tower in its present position; a lever arm extending 12.3 m from the tower centre and a load of 600 t would apparently provide the necessary counterforce. Alternative solutions to the engineering problem are however still being sought; and there is still no consensus on what actually caused the tilt. Perhaps the most ingenious theory is still that advanced by Kerisel (*Geotechnique* **25**, 433; 1975) who invoked the Coriolis force due to the rotation of the Earth. Is it possible, wondered Kerisel, that a structure with a very low safety factor might be influenced by sustained small forces acting over several centuries?

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and an alternative is the one mentioned earlier in the context of bullfinches, to provide a different food supply. These courses of action may, however, exacerbate the problem. If a

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pest species survives better as a result of the provision of refuges or alternative food supplies, it may return in great numbers in future years to attack agricultural crops. This kind of problem cannot be resolved without more knowledge of the factors determining survival and movements of bird pests. □

## One or more Eemian interglacials?

In a recent paper (*Nature* 277, 189; 1979) we described the Fjøsanger (interglacial) Stage in western Norway, and correlated it with deep sea oxygen isotope stage 5e and the Eemian of continental Europe. Bowen (*News & Views* 277, 171; 1979) accepted the former correlation but criticised the latter, because he agrees with Kukla's proposal (*Earth Sci. Rev.* 13, 307, 1977) that the Eemian represents three different interglacials.

The vegetational development in different interglacials may have been so similar that a separation on palynological evidence is impossible. However, this is not necessarily so, and the crucial point in the present discussion is whether other interglacials repeated the distinct pollen sequence so far being considered as the criterion for the Eemian.

We fully agree with Bowen's statement that the deep sea oxygen isotope stratigraphy proves that the classical subdivisions of the Quaternary of Europe are incomplete. That is our challenge! However, an incomplete continental stratigraphy does not imply that all previously defined units are ambiguous.

Bowen claimed that Weigank (*Geologie* 21, Beiheft 77, 1; 1972) has described two Eemian interglacials in superposition in northern Germany. On the contrary, however, he stated that these two interglacials can be identified on the basis of their pollen stratigraphy, the younger being the Eemian, and the older the Rügenian (=Kap Arkona in Frenzel's classification).

Most important Bowen stated that Kukla (*op. cit.*) has demonstrated that the classical Eemian sites in Europe were deposited during three different interglacials. No doubt, Kukla made an extremely interesting analysis of the Quaternary stratigraphy of Europe, but we cannot accept his evidence for more than one Eemian. Chiefly on the basis of geomorphological observations he postulated that the Saalian s.l. is composed of three distinct glacials, and he used Eemian sites for the presumptive interglacials so created. Most West German geologists (Düpphorn *et al.* *Eiszeitalter u. Gegenw.* 23/24, 222; 1973) reject the existence of an interglacial within the Saalian s.l., but

there are other more probable candidates (Rügen, Dömnitz/Wacken) to fill the milder intervals (see Weigank *op. cit.*; Erd *Palaeogeogr.*, -*clim.*, -*ecol.* 8, 129; 1970; Frenzel *Eiszeitalter alter u. Gegenw.* 23/24, 321; 1973; Menke & Behre *Eiszeitalter u. Gegenw.* 23/24, 251; 1973; Cepek *Ber. deutsch. Ges. geol. Wiss., A. Geol. Paläont.* 12, 375; 1967) than the Eemian, even if Kukla's interpretation is correct.

The main evidence in support of our thesis that the typical Eemian pollen sequence represents only one interglacial can be summarised as follows. Many known Eemian sites are located inside the Weichselian ice border; all of them are situated below till or disturbed by ice. More than 100 Eemian sites are known outside the Weichselian ice border, and, as far as we know, not a single sequence is reported to be below till, or below another interglacial. However, many 'not Eemian interglacials' are known below till outside the Weichselian ice border.

We conclude that our present knowledge strongly suggests that the Eemian pollen sequence of Europe represents only one interglacial, which is the Last Interglacial.

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D. Q. BOWEN REPLIES: The points raised by Mangerud *et al.* are only valid in so far as the chronostratigraphy advocated by Kukla and others is incapable of direct validation at present. But the fact remains that the system they advocate and defend in the case of 'Eemian' is based on assemblage floras, a biofacies basis inherently unreliable as a result of its diachronous nature. Indeed by advocating that Woillard's (*Quaternary Res.* 9, 1; 1978) St Germain Interglacials, I and II, are time-equivalent to interstadials in the Netherlands and Denmark, also urged by Wijmstra (in *Climatic Change* (ed. Gribbin) Cambridge, 1978), they tacitly acknowledge the fragility of this bio-

facies approach.

Discrimination of specific interglacials on the basis of either being covered by till or not in relation to postulated glacial limits is no improvement on 'count from the top' methods criticised by Kukla. In this way the current conventional wisdom, or model chronostratigraphic schema, is maintained. Data are not only interpreted on such model terms but are frequently acquired and systematised according to its pigeon-holed ready-made classification—the 'reinforcement syndrome' of the late Norman Watkins (*Comments on Earth Sci. Geophys.* 2, 36; 1971). In the case of Weigank's work the significant point relates to his demonstration that the foraminiferal faunas of the Eemian and Rügenian Interglacials show only minor differences.

The fact is that, according to present interpretation (Shackleton & Opdyke *Quaternary Res.* 3, 39; 1973) and defining the base of the Middle Pleistocene at the Brunhes-Matuyama boundary (Butzer *Quaternary Res.* 4, 136; 1974), some eight interglacials occurred from that time to the present. It is pertinent to note that one of the UK working parties on International Geological Correlation Programme Project 24 is examining evidence to determine how many interglacials there have been since the Hoxnian. Conventionally there is only one—the Ipswichian (Eemian)! In the search for greater complexity in continental records it is inevitable that some degree of data manipulation will occur initially because many of the basic facts regarding Pleistocene geology are still inadequately known. For too long has it been assumed that the Pleistocene geology of most formerly glaciated areas is known—perhaps it is, at least according to the terms of existing models. But in terms of modern lithostratigraphic standards (Hedberg *Int. Stratigraphic Guide*, New York, 1976) this work, with some notable exceptions (Willman & Frye *Illinois Geol. Surv. Bull.* 94, 1970), has hardly commenced. In the meantime, other than by rare geochronometric dating, progress will inevitably include some manipulation born out of an expediency dealing with inadequate data.

## A hundred years ago

We need not insist on the extreme importance and interest of the exhibition which was opened last night at the Albert Hall, and for which extensive preparations have been making for

some time. The public mind both in this country and abroad has been recently much agitated on the question of electric lighting, and, as might be expected, people are much confused among the many systems which have been brought forward, and even those

who know something on the subject must find it difficult to make up their minds. Hence the importance of bringing together the various systems of electric lighting in such a way as to make comparison possible.  
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# review article

## Facts and hypotheses of molecular chemical tunnelling

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*The similarities and differences between electron-nuclear tunnelling (discovered in 1966) and molecular tunnelling (discovered in 1973) are described here in terms of radiationless electron transitions. Examples of the low-temperature limit on a chemical reaction rate, observed since 1973, include: growth of chains of polymerisation of formaldehyde, Fe-CO rebinding in haem-containing proteins, isomerisation of radical pairs, hydrobromination of ethene, and photoinduced conversion of rhodopsin into prelumirhodopsin. The general significance of molecular tunnelling for chemistry and related subjects is discussed. The possible manifestations of molecular tunnelling in the formation of complex molecules at the surfaces and in the bulk of 'dirty ice' mantles of dust grains in interstellar dense clouds are also considered.*

ONE of the most important consequences of the wave properties of matter is tunnelling—the ability of particles to penetrate the potential barriers whose heights exceed the particles' kinetic energy; the sub-barrier region is therefore classically forbidden for such particles.

Tunnelling starts to become important when the de Broglie wavelength of a particle becomes comparable to the barrier width in a way that the probability of tunnelling decreases with an increase of the width ( $d$ ), the height ( $E$ ) of the potential barrier, and the mass ( $m$ ) of the tunnelling particle.

The tunnelling concept had its first successful application in nuclear phenomena such as  $\alpha$ -decay<sup>1,2</sup>, spontaneous fission, and thermonuclear reactions and later greatly contributed to solid state physics, electronics<sup>3-5</sup> and more recently to cosmology<sup>6</sup>.

### Chemical conversions

Such concepts in chemistry were initially concerned with the delocalisation of particles between two identical potential wells<sup>7</sup>, and led to analysing theoretically the tunnelling contribution to chemical reaction rates<sup>8-11</sup>. Such contributions should be particularly significant at low temperatures because—in accordance with Arrhenius' law—the rate of classical transitions over the barrier decreases exponentially with decreasing temperature while the probability of simple sub-barrier tunnelling should level off as  $T \rightarrow 0$ , rather than drop towards zero.

The quite general criterion of a 'tunnelling temperature'<sup>21</sup> is given by  $T_t = (\hbar/k_B \pi 2^{1/2} d)(E/m)^{1/2}$  where  $k_B$  is the Boltzmann constant. For  $T < T_t$  tunnelling starts to dominate exponentially the Arrhenius-type transitions and the rate of exothermic reactions in many common problems gradually reaches its low-temperature plateau.

Generally an exothermic transition  $i \rightarrow f$  requires the mixing of an initial level  $U_i^0$  with final levels  $U_f^n$  and the dissipation of the transition energy  $\Delta U_{if}$  provided by the fast electronic-vibrational relaxation of the final state (with the rate of such dissipation being larger than the rate of transition itself).

Different theoretical treatments lead either to the limit of the statistically averaged rate of such transitions or to their complete disappearance at low temperatures. Hence tunnelling does not necessarily produce the low-temperature plateau of chemical conversion rates, however, its occurrence is a very strong argument in favour of the quantum tunnelling mechanism of these conversions.

For several decades the search for chemical tunnelling was restricted to the region of comparatively large temperatures ( $T > T_t$ ), and conclusions about its existence were based on minor (up to tens of per cent) isotope effects in the rate of conversion of hydrogen-containing and deuterated compounds<sup>13</sup>.

However, even stronger kinetic isotopic effects in such conversions (up to factors of  $\exp(500/T)$ ) still do not confirm tunnelling because they can be caused simply by differences in zero-vibrational energies of Z—H and Z—D bonds.

Experimental observations of chemical tunnelling began in 1966 when Chance *et al.*<sup>14,15</sup> discovered the low-temperature (120–4 K) plateau (at  $W \sim 300 \text{ s}^{-1}$ ) of the reaction rate of the laser-flash initiated oxidation of cytochrome *c* by chlorophyll. Using the simplest model of tunnelling as being a penetration of a plane wave through the one-dimensional barrier and taking the pre-exponential factor as  $\omega_e \sim 4 \times 10^{15} \text{ s}^{-1}$  (Gamov factor  $G_t = (W/\omega_e) \sim 10^{-13}$ ) and the activation energy  $E \approx 0.14 \text{ eV}$  (as obtained from experiments up to 300 K) Chance *et al.*<sup>14,15</sup> estimated the tunnelling length of an electron as  $l \approx 30 \text{ \AA}$ . A similar value could be obtained by comparing the position of the low-temperature plateau with the tunnelling temperature  $T_t$  defined above.

Calculated tunnelling lengths of such an order of magnitude are also typical of many other examples of low-temperature transfer of electrons—in biopolymers and in irradiated frozen glasslike solutions—if they are treated simply using Gamov factors and assuming that the barrier height equals the activation energy in a high-temperature region.

However, such tunnelling resembles an electron current

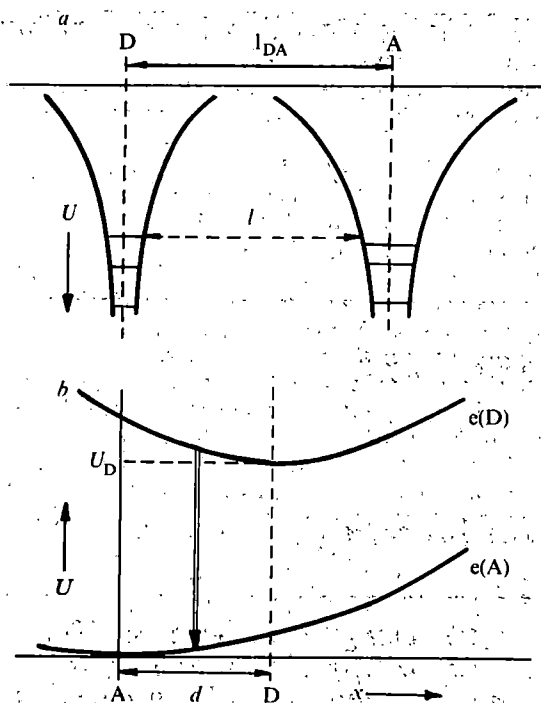


Fig. 1 Scheme of electron-nuclear tunnelling for electron transfer from the donor (D) to the acceptor (A). a, The tunnelling of electron over the distance  $l$  (levels in D and A do not usually coincide). b, The displacement of nuclei over the distance  $d$  for the transition of the electron  $e(D) \rightarrow e(A)$  indicated by vertical arrow.

through the thin dielectric layer which breaks the electron circuit rather than the transfer of reactants in the chemical reaction.

The general situation is much more complex because chemical tunnelling of electrons is related to the displacement of nuclei and we need to consider possible violations of the Franck-Condon principle. This was noted for the first time by Libby<sup>16</sup>, and was later analysed in several studies<sup>17-20</sup> of the tunnelling of electrons in oxidation-reduction interactions of ions in solutions and in electrode reactions.

After Chance's discovery<sup>14,15</sup>, when it seemed necessary to consider the electronic-vibrational interactions (vibrational coupling), the Franck-Condon factors in the tunnelling-type oxidation-reduction transformation of complex bimolecules were based on ideas of radiationless electron transitions (see refs 21-25). Different descriptions of electron tunnelling in biopolymers as generalised electron-nuclear tunnelling are described in refs 26-30.

In 1973 the discovery of a low-temperature limit of the rate of complex chemical reactions was first reported<sup>31,32</sup> using the example of the growth of polymerisation chains of formaldehyde, FA (see also refs 33-35).

We shall emphasise here that the words 'chemical reactions' describe transformations which include the reconstruction of molecules, the spatial rearrangement of atoms, changes in all main characteristics of valence bonds, their multiplicities and nature ( $\sigma$  or  $\pi$ -bonds) as well as lengths and angles.

The theoretical treatment of this phenomenon<sup>31,32</sup> as a new kind of molecular chemical tunnelling used the Siebrand's theory<sup>36</sup> of radiationless relaxation of electronically-excited molecular states. Later this theory was also extended to the case of molecular tunnelling, the so-called tunnel-polaron non-Arrhenius chemical reaction kinetics<sup>37</sup>.

Numerous surveys have been devoted to tunnelling of electrons in chemistry and biology (see refs 38-40) and we touch here only briefly the theory of such tunnelling to emphasise both the similarities and the differences of electron-nuclear and molecular tunnelling.

The probability of a radiationless electron transition  $W_{if}$  is determined by the product of the matrix element of the electron

transition  $L_e^2 = |\langle \psi_{ei} | \hat{L} | \psi_{ef} \rangle|^2$  and of the Franck-Condon factor  $F_v = |\langle \psi_{vi} | \psi_{vf} \rangle|^2$ :

$$W_{if} = (2\pi/\hbar) L_e^2 F_v \rho_f = \omega_{eff} F_v$$

where  $\psi_e$  and  $\psi_v$  are the electron and vibrational wave functions respectively,  $L$  is a transition operator such as the non-adiabaticity operator and  $\rho_f = (\hbar\omega_f)^{-1}$  is the density of vibrational levels in the final state.

The wave functions  $\psi_e(x)$  are proportional to  $\exp(-x/\alpha)$  for  $x > \alpha$ , where  $\alpha \sim \hbar/(2mE)^{1/2}$  is the damping parameter, that is the size of the region of localisation of electron density, which is close to the length of chemical valence bond  $a$  (at  $E \sim 1$  eV).

The wave functions  $\psi_v(x)$  are proportional to  $\exp[-(x/\Delta)^2]$  for  $x > \Delta$ , where  $\Delta = (\hbar/M\omega)^{1/2}$  is the amplitude of nuclear vibrations;  $\omega$  is the frequency of these vibrations;  $M$  the mass of the nuclei.

Among the most important parameters of electron and molecular tunnelling are the lengths of tunnelling (generally configurational displacement) given below as  $l$  for electrons and as  $d$  for heavy particles.

In most cases of electron-nuclear tunnelling in oxidation-reduction processes<sup>26-30</sup>:  $l \gg \alpha \approx a \gg d \approx \Delta$ .

The long-range transfer of electrons from the donor to the acceptor is not accompanied by chemical reaction. Displacements of donor and acceptor nuclei proceed in a similar way to ordinary intramolecular radiationless transitions up to distances of the order of  $\Delta \sim 0.1$  Å (see Fig. 1).

In the case of molecular tunnelling in chemical reactions we have  $d \approx l \approx a \gg \Delta$ .

For sufficiently large electron ( $l \gg \alpha$ ) and nuclear ( $d \gg \Delta$ ) displacements, when the matrix elements of the transition probability are determined by the asymptotics of wave functions, the matrix element of the electron transition transforms into the ordinary Gamov-type tunnelling factor, that is  $L_e^2 \propto \exp[-\psi l(mE)^{1/2}/\hbar]$  ( $m$  is the electron mass,  $\psi \sim 1$  depends on the shape of the barrier), while the vibrational (nuclear) Franck-Condon factor depends exponentially on the square of the nuclear displacement:  $F_v \propto \exp[-(d(M\hbar\omega)^{1/2}/\hbar)^2]$  (here  $\omega = \omega_i \ll \omega_f$ )<sup>35</sup>.

Parameters of various processes interpreted in terms of radiationless electron transitions are summarised in Table 1.

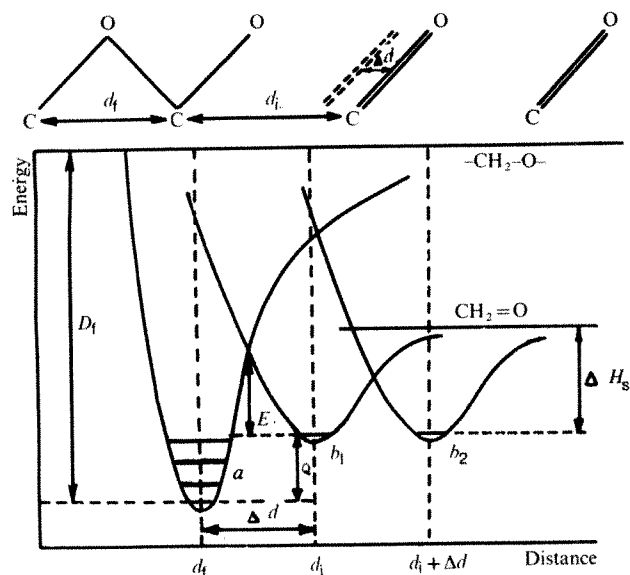
Just as Chance's discovery<sup>14,15</sup> is generally regarded as a first experimental proof of the existence of chemical electronic tunnelling, various examples of a low-temperature plateau of the rate of chemical reactions can be considered as experimental confirmation of the existence of molecular chemical tunnelling.

The first such example as mentioned above was the discovery of a limit (below  $\sim 12$  K) of the rate of growth of chains in

Table 1 General characteristics of processes interpreted in terms of radiationless electron transitions

	$l$	$\omega_{eff}/\omega_0^*$	$d$	$F_v$	$W_{exp}(s^{-1})$
1. Electron-vibrational relaxation	$\sim \alpha$	$> 10^{-3}$	$\sim \Delta$	$> 10^{-3}$	$10^{14}-10^8$
2. Tunnelling of electrons in oxidation-reduction conversions:					
(i) not taking into account the nuclear displacement;	$(10-30)\alpha$	$< 10^{-8}$	0	1	$10^6-10^{-2}$
(ii) taking into account the electronic-vibrational coupling	$< 10\alpha$	$< 10^{-5}$	$\sim \Delta$	$> 10^{-3}$	
3. Molecular tunnelling in chemical reactions:					
(i) 'pure' molecular tunnelling;	$\sim \alpha$	$> 10^{-3}$	$\sim a$	$< 10^{-4}$	$10^{10}-10^{-4}$
(ii) tunnel-polaron transitions	$\sim \alpha$	$> 10^{-3}$	$d_i \sim \Delta$ $\sum d_i \sim a$	$> 10^{-3}$	

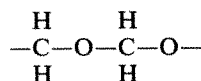
\*  $\omega_0^* = 10^{14} s^{-1}$  is a typical value of pre-exponential factor in chemical reactions.



**Fig. 2** The upper simplified scheme shows positions and displacements of molecules of solid formaldehyde and the polymer chain. The lower scheme illustrates the tunnel transitions of  $\text{CH}_2\text{O}$  groups during polymerisation ( $b_1 \rightarrow a$ ) and during filling up of 'vacancies' formed near the end groups of polymer chain during polymerisation ( $b_2 \rightarrow b_1$ ). The curve  $a$  is the adiabatic curve for a formaldehyde molecule added to a growing polymer chain;  $b_1$  is a similar curve for the initial monomer molecule, and  $b_2$  represents a similar curve for the next monomer molecule (the zero on the  $x$ -axis for this curve has been shifted by a distance  $\Delta d$ ). The energy of dissociation of the C-O bond is  $D \approx 4$  eV, energy of the sublimation of crystalline FA is  $\Delta H_s \approx 0.3\text{--}0.4$  eV, polymerisation heat  $Q \approx 0.37$  eV, activation energy  $E = 0.1$  eV. Note that the product  $\Delta d(M_{\text{CH}_2\text{O}}E)^{1/2}$  is close to the corresponding value for spontaneous fission of atomic nuclei, where  $\Delta d \approx 10^{-12}$  cm and  $E \sim 1$  MeV.

radiation-induced polymerisation of solid formaldehyde<sup>31-35</sup> studied by calorimetric method between 140 and 4 K.

Stacks of planar triangles of monomer  $\text{CH}_2\text{O}$  molecules with double  $\text{C}=\text{O}$  bonds transform in this reaction into long chains of single tetrahedral



bonds, and the substitution of van der Waals radii of C and O (typical for monomers) by much shorter valence  $-\text{C}-\text{O}$  bonds in polymers leads to a considerable ( $\sim 40\%$ ) increase in density. The scheme used for the calculation of the tunnelling rate in formaldehyde polymerisation is illustrated by Fig. 2.

In such an analysis the rate of growth of the polymer chain depends exponentially on the square of the molecular tunnelling distance  $(\Delta d)^2$  of the monomer group. Besides the addition of new links to the growing chain, the polymerisation includes in this calculation the subsequent stage of delocalisation of the cavity formed at the border between the end link of the polymer chain and the neighbouring monomer molecule—provided, for example by quantum diffusion<sup>41-43</sup>.

Another variant shown in Fig. 3 is the replacement of a well-defined cavity between the polymer and monomer by an extended distorted region of variable density (such as the stretched end of a polymer chain or the 'swollen' monomer that fills the cavity) which plays to some extent the part of polaron-deformed region of the crystal and is transferred (following the transfer of 'driving' particle such as an electron) with the end of the growing chain, corresponding to the above mentioned tunnel-polaron variant of non-Arrhenius kinetics<sup>37</sup> listed—among the other mechanisms—in Table 1.

Adopting a complex (generally, multidimensional) picture of potential surfaces means we can consider the possibility of conversions through low barriers: in other words, the possibility of eliminating various restrictions on the tunnelling rate which

are in ordinary molecular tunnelling due to large masses or large displacements of reacting species.

The temperature dependence of the rate constant on the growth of polymerisation chains of formaldehyde is shown in Fig. 4.

The average time of adding one new link to the growing polymer is  $\tau_0 = k^{-1} = \tau/G$  (where  $\tau$  is the time taken for the growth of the whole chain and  $G$  the radiation-chemical yield of polymerisation) which is  $\tau_0 \approx 10^{-2}$  s at the plateau.

Frauenfelder *et al.*<sup>44-45</sup> confirmed the existence of molecular tunnelling in a study of low-temperature Fe-CO reconstitution in a haem-containing protein and protohaem. Fe-CO bonds were broken by lasers flashes and the kinetics of their reconstitution was spectrophotometrically followed over very wide intervals of time ( $10^{-6}$ – $10^3$  s) and temperatures (2–340 K).

Besides finding a low-temperature plateau below  $\sim 10$  K (see Fig. 4) these observations demonstrated the complex, polychromatic character of these kinetics, that is the existence of broad spectra of activation energies and barrier widths rather than single values of these parameters.

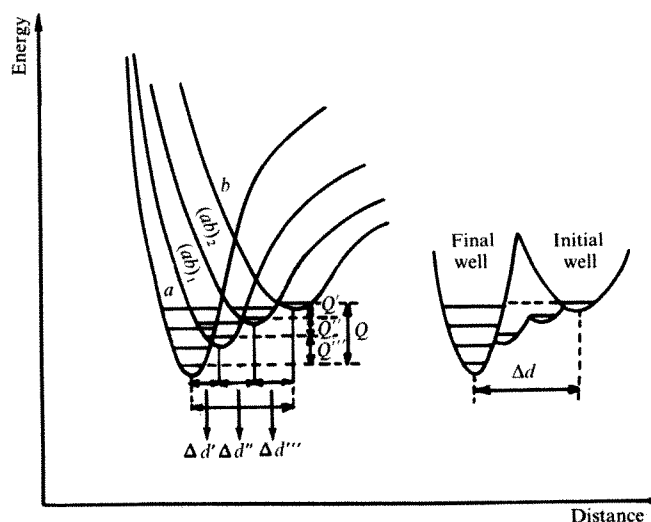
The polychromatic kinetics described in refs 48–49 and later combined with the tunnelling concepts<sup>50,51</sup> is typical for systems with many different reactant states represented, for example, by different conformers of proteins or by traps of different depths in irradiated molecular solids.

Frauenfelder *et al.*<sup>44-47</sup> successfully used polychromatic kinetics to solve the complex picture of several potential barriers hindering Fe-CO reconstitution and to correlate these barriers with the structures of protohaem, myoglobin and haemoglobin.

The low-temperature rate limit of the processes studied by Frauenfelder *et al.* was also demonstrated by Mössbauer absorption spectroscopy<sup>52,53</sup>.

Meanwhile using Mössbauer emission spectroscopy (in particular, on combining it with the technique of delayed  $\gamma\gamma$ -coincidences) allowed electron-nuclear tunnelling to be observed which caused the change of state of metallic ions in iron-cyanide<sup>54</sup> and cobalt-phenantrolyne<sup>55</sup> complexes.

A Japanese research group<sup>56</sup> has recently extended the earlier ESR observations<sup>57</sup> of conversions of radicals in  $\gamma$ -irradiated dimethylglyoxime at 77–220 K to lower temperatures (4–77 K) and succeeded in finding the low-temperature plateau of the rate of the comparatively slow process of isomerisation of radical pairs (see Fig. 4) which is characterised at higher temperatures



**Fig. 3** The adiabatic potential curves for the normal ( $a$ ) and 'stretched' ( $b$ ) groups of polymer chains and  $\text{CH}_2\text{O}$  molecules in the crystal of a monomer. In the case of a well-defined hole the curves  $a$  and  $b$  represent two neighbouring states separated by a potential barrier (shown in the figure) which prevents elementary chain growth. In the case of a 'stretched' polymer chain there are two possible intermediate states:  $(ab)_1$  and  $(ab)_2$  between  $a$  and  $b$ . Any barriers are absent in transitions of the type  $b \rightarrow (ab)_2 \rightarrow (ab)_1 \rightarrow a$ .



by an activation energy  $E \approx 0.65$  eV. Having studied the kinetics of hydrobromination for an equimolar ethene-HBr mixture between 90 and 30 K, our group observed long ( $\nu \sim 200$ ) chains of this reaction even<sup>34,58</sup> at 30 K. Subsequent calorimetric investigations of stationary and non-stationary kinetics of chain hydrobromination in the conditions of predominantly quadratic or linear rupture of chains led<sup>90</sup> to the numerical determination of the rate constant of propagation of chains ( $k_p = \tau_0^{-1} = G/\tau$ ) and to finding another example of the limit of the rate of elementary chain reactions at low temperatures (see Fig. 4).

Application of picosecond laser spectroscopy to the studies of photo-initiated rhodopsin-prelumirhodopsin conversion<sup>59</sup> has demonstrated once more a low-temperature reaction rate plateau.

However, there is no agreement about the mechanism of this process: there exist two main approaches, namely the tunnelling of one or two hydrogen atoms to the nitrogen or Schiff base (across a distance of 0.5–0.9 Å)<sup>59</sup> or the low-temperature *cis-trans* isomerisation of the chromophore group (retinal) bound to the protein (opsin)<sup>60–62</sup>.

The large reaction rate at the plateau ( $K_H \approx 2.8 \times 10^{10} \text{ s}^{-1}$ ) and a quite weak kinetic isotopic effect ( $K_H/K_D \approx 7$ )—see Fig. 4—remind us of the possibility of a tunnel-polaron mechanism<sup>37</sup> of low-temperature isomerisation—via several states represented by intermediate potential curves in Fig. 3. Confirmation of this idea would be highly significant to the problems of low-temperature quantum chemistry, therefore, it is very important to make a definite choice between the transfer of hydrogen atoms in rhodopsin and the isomerisation of retinal.

The tunnelling of atoms and molecules with a rearrangement of chemical bonds should not be treated as exclusive to low-temperature chemistry. Such tunnelling can also be observed as sub-barrier predissociation of molecules by very precise laser tuning of their excitation energy below the Landau-Zener gap (this possibility was mentioned in ref. 38) or by electron

beams<sup>63</sup>. Isotopic differences in the probabilities of such tunnelling may be useful for isotope enrichment.

One of the new topics of macroscopic chemical kinetics which could result from tunnelling is the cold initiation of thermal explosions by high pressures which enhance the tunnelling as well as the possibility of oscillations between subcritical and overcritical states of the system compared with the conditions of thermal explosion.

It is also possible that in some cases the usual transition from the diffusion to the kinetic region with decreasing temperature will be supplemented by a return to the diffusion region below the tunnelling temperature—because of the gradual disappearance of temperature dependence on the rate of the kinetic stage of conversion.

The high selectivity of low-temperature tunnelling reactions caused by the interplay of the three main parameters— $d$ ,  $M$  and  $E$  may be advantageous for applied cryochemistry and cryobiology.

The persistence of chemical reactivity even near absolute zero makes it doubtful whether a complex organism could be brought back to life after being subjected to prolonged freezing in conditions when chemical chain reactions might be initiated by external agents like radiation.

Of special interest is the possibility of chemical and prebiotic evolution at very low temperatures when exothermic reactions (including the formation of highly complex products) are thermodynamically favoured (because all entropy restrictions are eliminated as  $T \rightarrow 0$ ) and at the same time—in spite of the Arrhenius law—quantum effects permit measurable, finite rates of chemical conversions.

The problem of cold tunnelling evolution from the 'storage of negative entropy' up to prebiotic levels has been discussed previously<sup>30–34,64–66</sup>. The possibility of tunnel-polaron-type<sup>37</sup> low-temperature exothermic reactions of various kinds (such as polymerisation and polycondensation) could be particularly significant.

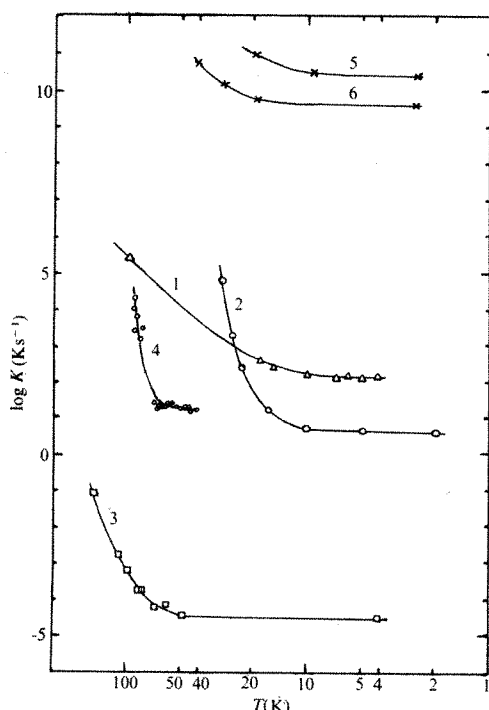
## Interstellar chemistry

In view of all these circumstances we now consider the role of low-temperature quantum effects of chemical kinetics in the formation of complex products of interstellar chemistry. However, we should emphasise that—in contrast with the experimental facts and theoretical considerations described above—the following comments are purely hypothetical and they are intended to stimulate discussions and new experiments. The problems of interstellar chemistry have been treated in many recent surveys (see refs 67–77, 91), and we will consider them only to the extent needed for discussing the possibilities of molecular tunnelling in interstellar chemical reactions.

The most abundant element in the Universe is hydrogen. The cosmic abundance of helium (relative to hydrogen) is 0.09, followed by oxygen ( $7 \times 10^{-4}$ ), carbon ( $3 \times 10^{-4}$ ), nitrogen ( $9 \times 10^{-5}$ ), then magnesium, silicon and iron ( $\sim 10^{-4}$  altogether). Amongst almost 50 interstellar molecules detected by radio and optical spectroscopy the most widespread apart from  $\text{H}_2$  are: CO ( $\sim 10^{-4}$ ),  $\text{NH}_3$ , HCN and HNC ( $\sim 10^{-6}$ ). There is no doubt that water and methane are also well represented in interstellar space, and we should also mention formaldehyde ( $\sim 10^{-8}$ ) whose low-temperature polymerisation was directly observed in the laboratory.

The main stage of interstellar chemistry is the multitude of so-called interstellar clouds—giant ( $10^{17}$ – $5 \times 10^{19}$  cm) suspensions of dust grains in a gas medium. The total dust amounts to only about 1% of the gaseous mass; dust grains are generally assumed to consist of central 'cores' ( $r \geq 10^{-6}$  cm) of silicates or graphite surrounded by mantles of 'dirty ice' ( $r \sim 10^{-5}$  cm) formed by a mixture of frozen substances. The average number of hydrogen atoms per dust grain in the cloud is  $10^{12}$ .

One of the main differences between diffuse ( $n_{\text{H}} \sim 10$ – $10^3 \text{ cm}^{-3}$ ) and dense or dark ( $n_{\text{H,H}_2} \sim 10^3$ – $10^7 \text{ cm}^{-3}$ ) clouds is the following: diffuse clouds are transparent to UV radiation which causes ionisation of atoms and dissociation of molecules, while



**Fig. 4** Summary of data on the low-temperature limits of rate constants ( $\text{K s}^{-1}$ ) of chemical reactions ( $\log K$ – $\log T$  coordinates). Curve 1 ( $\Delta$ ), growth of chains of polymerisation of formaldehyde. Curve 2 ( $\circ$ ), reconstitution of Fe–CO bonds in  $\beta$ -haemoglobin. Curve 3 ( $\square$ ), isomerisation of radical pairs in  $\gamma$ -irradiated dimethylglyoxime. Curve 4 ( $\circ$ ), propagation of chains in the hydrobromination of ethene. Curve 5 ( $\times$ ), photoinduced conversion of rhodopsin into prelumirhodopsin. Curve 6 ( $\times$ ), photoinduced conversion of deuterated rhodopsin into prelumirhodopsin.

dense clouds are opaque to UV radiation. Therefore photodissociation does not proceed in the depths of dark clouds and a much wider variety of complex,  $m$ -atomic molecules (up to  $m = 11$ , cyanotetra-acetylene  $\text{HC}_9\text{N}$ ) are present here. This is why dark clouds are also referred to as molecular clouds. The temperature of dust grains in these clouds is  $T = 10\text{--}20\text{ K}$ .

The outer regions of dark clouds are transparent to UV radiation (its flux  $\mathcal{F}_{\text{UV}} \sim 3 \times 10^8 \text{ cm}^{-2} \text{ s}^{-1}$  and its cross-section for interaction with molecules is  $\sigma_{\text{UV}} \sim 10^{-18} \text{ cm}^2$ ), and each molecule absorbs a UV quantum within  $\tau_{\text{UV}} \sim 100 \text{ yr}$ .

The only external agents which can ionise or excite molecules inside the clouds are energetic cosmic protons. They interact with each interstellar molecule within  $\tau_p \sim 3 \times 10^9 \text{ yr}$ . This value strongly exceeds not only  $\tau_{\text{UV}}$ , but also the lifetime of the clouds themselves which has been determined by their collisions and by gravitational collapse to be  $\tau_{\text{cl}} \sim 10^5\text{--}10^7 \text{ yr}$ .

In discussing interstellar chemistry we need to consider three types of chemical reactions—in the gas phase, at the surfaces of dust grains, and in the bulk of dirty ice. Tunnelling plays no part in gas phase reactions and they will not be considered here.

As far as reactions at the surfaces of dust grains are concerned, most attention has been paid to the activationless recombination of free atoms and radicals<sup>69,70,75</sup>. According to existing estimates<sup>70</sup> tunnelling can play an important and even decisive part in lateral diffusion processes which precede such recombination for physically and chemically adsorbed hydrogen atoms and for heavier atoms and radicals in the case of their physical adsorption.

What this means to molecular tunnelling (or tunnel-polaron transitions) as a possible mechanism of chemical reactions at the surface and in the bulk of dust grains, has been discussed recently<sup>64,65</sup>. An analysis of the possible role of tunnelling in such conversions should take into account the competition between the chemical reactions of complex molecules (components of dirty ice), their ejection from surfaces into the gas and their destruction by interstellar radiation which can not only decompose more complex molecules into simpler species but also initiate exothermic chain reactions of molecular combination for example, polymerisation. The destruction effects of radiation seem to restrict the formation of complex,  $m$ -atomic molecules in gas-phase reactions and in the recombination of radicals at grain surfaces to  $m \sim 10\text{--}12$ . Therefore such reactions cannot account for the formation of more complex interstellar molecules—up to polymer size.

Meanwhile astrophysicists such as Sagan<sup>73,76</sup>, Greenberg<sup>72,77,78</sup>, Wickramasinghe<sup>79,80</sup> and Hoyle<sup>81–83</sup>, although differing in their conjectures and conclusions about the nature of very complex interstellar molecules and their origins, nonetheless agree that such molecules do exist in the interstellar dust. These conclusions are based both on laboratory analyses of products of low temperature UV illumination of solid mixtures resembling the dirty ice<sup>73,77,78</sup> (however, these analyses were performed after defrosting such mixtures, and therefore indicated the products of not only the 'cold' reactions but also of their after effects) and on the comparison of galactic absorption and emission IR spectra with the laboratory spectra of trial compounds<sup>79–83,76</sup>.

Sagan considers the tar-like macromolecular compounds of irregular composition and structure formed in his laboratory model experiments as likely interstellar compounds and called<sup>76</sup> them tholins.

After comparing various terrestrial and interstellar IR spectra Hoyle and Wickramasinghe arrived at some interesting although controversial conclusions about the formation of interstellar polymers of formaldehyde, of polyoxymethylene<sup>79,80</sup> and even of polysaccharides<sup>81–83</sup>.

All these authors agree that the formation of any polymer requires the growth of macromolecules in a chain reaction, but they differ in the astrophysical conditions deemed necessary for such chain reactions.

According to Sagan<sup>73,76</sup>, polymers and other sufficiently thermostable complex organic substances were transported into

the interstellar gas as components of grains ejected from solar nebulae by radiation pressure and stellar winds at the early stages of stellar evolution. Furthermore, tens of identified interstellar  $m$ -atomic molecules ( $m = 2\text{--}11$ ) were formed not in the usual processes of integration in the gas phase and at the surface of grains but as degradation products of more complex interstellar dust compounds. Although we shall not discuss this point of view here, it should be noted that polymers above a certain temperature  $T_d$  are quite often not truly stable but only metastable, when stabilised by endcapping groups (see ref. 84). Being formed below  $T_d$  such polymers remain stable even above  $T_d$ , but polymer chains will not grow at  $T > T_d$ .

Another idea is that the polymerisation (as well as the formation of complex molecules by recombination of radicals<sup>69,70,72,75,77,78</sup>) proceeds directly in the interstellar dust grains at  $T = 10\text{--}20\text{ K}$ , in the bulk of the ice mantles or at their surfaces. Even in the depths of dense clouds polymerisation chains can be triggered by long-range cosmic protons ( $E_p \geq 100 \text{ MeV}$ ). However, before the discovery of the low-temperature limit of the chemical reaction rate speculations about the hypothetical formation of polymers within dense clouds lacked kinetic grounds.

At present we can attempt quite a detailed analysis of such possibilities for at least one example, the polymerisation of formaldehyde.

## Interstellar polymerisation

Let us start with the abundance of formaldehyde in dirty ice. The saturated gas phase concentration of CO in equilibrium with solid carbon monoxide at 20 K is  $\sim 10^5 \text{ cm}^{-3}$ , and for methane  $\sim 1 \text{ cm}^{-3}$ . These values are larger or fairly close to the real concentrations of CO and  $\text{CH}_4$  in the interstellar gas which, in such circumstances, can be undersaturated. Therefore the freezing-out of  $\text{CH}_4$  (and especially of CO) seems to have much less significance on the formation of dirty ice than the solidification of ammonia, HCN, HNC, water and ethane.

Characterising the volatility by the temperature which corresponds to an equilibrium gas concentration of  $1 \text{ cm}^{-3}$ , then we have in order of decreasing volatility: ethane ( $\sim 35 \text{ K}$ ), ammonia and formaldehyde ( $\sim 60 \text{ K}$ ), HCN and HNC, then water ( $\sim 90 \text{ K}$ ) and, finally, a polymer of formaldehyde–polyoxymethylene ( $\sim 100 \text{ K}$ ). The rate of condensation does not depend on volatility while the rate of sublimation (both spontaneous and radiation-induced) decreases rapidly with diminishing volatility. Therefore these consequences accord with the steady-state enrichment of dirty ice by various components in comparison with gas. While the relative abundance of formaldehyde in the gas phase is no larger than several tenths of a per cent of all the other listed compounds, its abundance in the solid phase, in its polymer form, could be much higher. Moreover, we should consider that the above listed cosmic abundances of various elements provide only for water and formaldehyde the possibility to form the whole mass of dust grains (0.01 of hydrogen mass) while the maximum total mass of interstellar ethane is half of that, of HCN and of HNC a quarter of that, and of ammonia a sixth of that.

The attainment of steady-state conditions requires perceptible gas-solid transitions in both directions, that is a combination of condensation and sublimation. The condensation rate is high enough: the number of molecules of a certain kind condensed at each grain during the cloud life-time  $\tau_{\text{cl}}$  (years) is  $100 \kappa n \tau_{\text{cl}} (T/M)^{1/2}$ , where  $M$  is their molecular mass (in daltons);  $\kappa n \text{ (cm}^{-3}\text{)}$  their gas phase concentration ( $n = n_{\text{H}, \text{H}_2}$ ,  $\kappa \leq 10^{-6}$ );  $\epsilon \leq 1$  their sticking probability. For the most dense and long-lived clouds ( $n \tau_{\text{cl}} \geq 10^{12}$ ) the condensation can even lead to a several-fold increase of the masses of grains during the time that the cloud exists.

Estimates of the sublimation rate contain more uncertainties. The rate constant of spontaneous (thermal) evaporation of molecules bound to the surface with a binding energy  $D$  equals  $K_{\text{se}} \sim 10^{12} \exp(-D/k_B T)$ , and the time of such an evaporation of the surface monolayer would exceed the clouds

lifetime if  $(D/k_B) \geq 60 T$ .

Detailed estimates of the rate of induced evaporation caused by the transient heating of the entire grain by low-energy cosmic rays and, to a lesser extent, by the spot heating of the same origin are given in ref. 70.

According to these estimates the lifetime of clouds ensures numerous repetitions of evaporation of molecules physically adsorbed at the strange lining (at  $D \approx 0.05\text{--}0.1$  eV). However, the evaporation of mantle would be strongly hindered by its conversion to a crystal with greater binding energy. There will be no evaporation at all of molecules incorporated into the polymer chain. Therefore one can expect formaldehyde to be more abundant in the surface layers of interstellar grains because of its polymerisation.

The radiation-chemical yield of solid-state polymerisation of crystalline formaldehyde at 20 K is  $G \sim 10^4$  molecules per 100 eV of radiation energy<sup>31-35</sup>. Thus the energy delivered by cosmic protons to a grain during  $\sim 3 \times 10^5$  yr is already sufficient to cause its complete polymerisation if it were to consist exclusively of formaldehyde: mixed composition of dirty ice certainly hinders polymerisation. Nevertheless, although the active end group is surrounded from all sides but one by admixtures it will 'automatically' select the only possible direction to add the neighbouring monomer molecule. The formation of a new link of the growing chain at the surface can be preceded by several futile encounters of the end group with other molecules hitting the surface from gas phase or diffusing along the surface. It would be interesting for interstellar chemistry to compare the  $G$  values for low-temperature polymerisation initiated by electrons and  $\gamma$  rays (when the local heating along the tracks is insignificant), by protons, and by heavy ions (when the heating along the tracks is particularly strong<sup>85</sup>). It would also be desirable to determine directly the radiation yield of the evaporation of various molecules from different surfaces.

The rate of lateral diffusion of physically adsorbed molecules at low temperatures is determined by tunnelling and an estimate<sup>70</sup> of the time of a quantum jump of a molecule with  $M = 30$  gives  $t_{tr} \sim 0.1\text{--}1$  s. For the diffusion in the 'strange matrix' when the encounter of reactants requires  $N$  jumps, the reaction rate would be determined either by diffusion (at  $\tau_0 < t_{tr}$ ), or by kinetic factors (at  $\tau_0 > t_{tr}$ ), and the time of reaction would correspondingly be the time of  $N$  or of  $(N\tau_0/t_{tr})$  jumps. If the end group of a growing chain is surrounded only by its own monomer, the diffusion factors cease to play any part, and the possibility or impossibility of effective polymer accumulation in the interstellar dust grains is determined by the interplay of four characteristic times— $\tau_0$ ,  $\tau_{uv}$  (for diffuse clouds and outer layers of dense clouds), and  $\tau_{cl}$  (deep in dense clouds), as  $\tau_{cl} < \tau_p$ .

Consideration of these criteria leads to the following inequalities required for the realisation of chain reactions with chain length  $\nu$ , in dense clouds:

$$\tau_0 \ll (10^{-3}\text{--}10^{-2})\tau_{uv}/\nu \quad \text{or} \quad \tau_0 \ll \tau_{cl}/\nu$$

Taking the value of the pre-exponential factor  $\omega_0 \approx 10^{14} \text{ s}^{-1}$  one gets the following condition for the classical (Arrhenius-type) interstellar chain reaction to proceed:  $E < T(5.6 \times 10^{-3} - 2 \times 10^{-4} \log \nu)$  eV.

In the case of polymerisation of formaldehyde ( $\nu \geq 10^3$ ,  $E_{exp} \approx 0.1$  eV, refs 31-35) the inequality  $\tau_0 \ll 10^2\text{--}10^4$  yr would be fulfilled for Arrhenius kinetics only at  $T > 20$  K, while the experimental value is  $\tau_0 \approx 10^{-2}$  s, at  $T = 4\text{--}12$  K.

An approximate representation of the rate of tunnelling is obtained by using Gamov factors (see refs 37, 66):  $G_t = \exp(-\beta d(mE)^{1/2}/\hbar)$ . This yields the following condition for quantum (tunnelling) chain polymerisation of formaldehyde in dense clouds:

$$\beta d(mE)^{1/2}/\hbar < 4 - 0.15 \log \nu$$

where  $\beta = 2\sqrt{2}$  for a rectangular barrier,  $\beta = \pi\sqrt{2}/2$  for a parabolic barrier, and  $\beta = 4\sqrt{2}/3$  for a triangular barrier,  $d$  is in angstroms, and  $E$  in electron volts. This inequality corresponds to:  $d(E)^{1/2} \leq 60\text{--}90$  for the tunnelling of electrons,  $d(E)^{1/2} \leq 1.4\text{--}2$  for H atoms,  $d(E)^{1/2} \leq 0.35\text{--}0.5$  for formaldehyde mole-

cules in a monomer matrix.

In this way tunnelling could significantly increase the number of possible low-temperature reactions in dense clouds. One case would be, for example, the possibility of tunnelling polymerisation at the surface of dust grains with the formation of a very thin (several molecular layers) polymer film around the inner region of dirty ice. Such a film could protect the surface of this inner region from both condensation and sublimation.

For chemical and prebiotic evolution the reactions of polycondensation in dirty ice mantles with the participation of  $\text{CH}_2\text{O}$ , HCN, HNC,  $\text{NH}_3$  and  $\text{H}_2\text{O}$  are of interest. Such reactions lead to the formation of amino acids, polypeptides, sugars and nucleotide bases (purines and pyrimidines), they are exothermic, but not chain-type.

There are no reasons why there could be a 'pure' molecular tunnelling mechanism of such reactions—the rate of tunnelling falls steeply to a vanishingly small limit with increasing barrier widths and masses. However, each single step of a chemical conversion which represents an elementary gas phase process (such as the reaction  $\text{H}_2\text{C}=\text{O} + \text{NH}_3 \rightarrow \text{H}_2\text{C}=\text{NH} + \text{H}_2\text{O}$ ), proceeds in the solid as a sequence of many individual or collective conformational rearrangements of molecules, complexes or indeed whole regions of molecular crystals. The collision of a dust grain with a cosmic proton or UV quantum, or the release of recombination energy at the grain surface can induce the transfer of the 'driving' particle, such as the electron, which determines the number of conformational rearrangements. In this way the tunnel-polaron mechanism<sup>37</sup> becomes involved: the single long-range transfer of a molecule ( $d \approx a$ ) is replaced by several short-range displacements ( $d_i \approx \Delta$ ,  $\sum d_i \approx a$ ) which can lead to the lowering or even to the disappearance of the activation barrier (see Fig. 3), and to a reasonably high and temperature-independent rate of chemical conversions of cold systems such as of isomerisation. This mechanism could be important also for chemical and prebiotic evolution.

One should, however, bear in mind that if a certain product is formed as a final step in a non-chain sequence of  $\eta$  chemical conversions, and each of these conversions needs an external stimulus, then the yield of such product would be  $\eta\nu$  times smaller than the number of molecules entering the chain of length  $\nu$ . Therefore the yield of multistep polycondensation reactions accumulated during the life-time of dense clouds would not exceed 0.01% of the mass of the grains.

The formation of even the most complex biopolymers in interstellar dust grains does not guarantee their conservation during the later formation of new stars and planetary systems, when the dense clouds collapse. One can not exclude, for example, the strong heating at the surfaces of planets at certain stages in their evolution. Nevertheless, one should also keep in mind the variety of possible chemical reactants and reactions in 'warm' systems depending on their history. For example, polymers created and stabilised by endcapping at low temperatures could survive the consequent warming, but they cannot be formed directly at higher temperatures. Meanwhile the existence of such polymers opens additional possibilities of chemical conversions not only in cold, but also in warm systems, such as the integration of molecules in shock-wave induced solid-phase reactions<sup>86,87</sup> and in the vicinity of phase transitions, which proceed during the alternate heating and cooling of reactants<sup>88,89</sup>. Favourable conditions for the latter type would be provided, for example, by multiple transportation of stable organic compounds between circumstellar disks and interstellar clouds<sup>76</sup> and in comets with extended orbits which alternately suffer short heating when approaching the Sun, and prolonged deep cooling, away from the Sun.

The hypothesis of a cold 'entropyless' exothermic formation of complex molecules permitted only by quantum chemical reactivity is not an alternative, but an additional idea on the mechanisms of chemical and prebiotic evolution in terrestrial and extraterrestrial conditions.

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## articles

# New large-scale magnetic features of the Milky Way

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*A large body of newly determined rotation measures (RM) for extragalactic radio sources gives a new, much clearer picture of the magnetic structure of our Galaxy. The RM sky is dominated by a very strong zone which extends to large (down to  $b \approx -40^\circ$ ) negative galactic latitudes. Both the strength and extent of this feature are greater than appreciated from earlier data. It coincides in angular position with loop II of the non-thermal radio background radiation of the Galaxy. This, and two other strong RM features suggest that our Galaxy has very large-scale magnetic field zones, probably between the spiral arms, and that the prevailing sense of the field is opposite in the interarm regions on each side of the Sagittarius arm.*

WE report the discovery of some new large-scale features of the galactic magnetic field which have been obtained from a programme to determine accurate rotation measures (RM) of a large sample of extragalactic sources.

Previous analyses of Faraday rotation<sup>1-7</sup> have shown that a systematic modulation of the RMs occurs with galactic coordinates ( $l, b$ ) and that RMs are systematically larger at low galactic latitudes. They also indicate a prevailing sense of RM consistent with a local magnetic field directed towards  $l \approx 90^\circ$  (refs 2, 4, 6, 7), a trend which also agrees with that deduced from the RMs of pulsars<sup>8</sup>.

Our new polarisation data have greatly improved the number and quality of RM determinations for extragalactic radio sources, and currently give 528 RMs. This is nearly a factor of 2 more than the number of previously published RMs of equivalent reliability. The increased density of sources on the

galactic ( $l, b$ ) sky provides us with the clearest picture yet obtained of the large-scale galactic contribution to the RMs.

### A large, high-latitude anomaly in the RM sky

Figure 1 shows the distribution in galactic coordinates of rotation measures of 476 extragalactic sources. This sample has been purged of 52 sources whose RMs, although accurately determined, are likely to be 'contaminated' by Faraday rotation in the sources themselves, rather than in the interstellar medium. We have established that sources with high depolarisation rates (that is, low  $\lambda_{1/2}$  values; see ref. 9) have systematically higher RMs. We have therefore removed sources with low  $\lambda_{1/2}$  ( $< 10$  cm), as this is an independent source parameter unlikely to be related to the effects of our Galaxy.

The most prominent feature in the RM sky is a large zone of negative RMs from  $b = +10^\circ$  to  $-40^\circ$  at  $45^\circ \leq l \leq 160^\circ$ . We shall denote this as region A. Although previous RM data showed the region centred near  $l \sim 90^\circ$  to have predominantly negative RMs, the large angular scale and strength of this region are revealed for the first time by our new data. Figure 1 shows that surprisingly large RMs, up to  $200 \text{ rad m}^{-2}$ , occur at negative latitudes as far as  $-30^\circ$  near  $l \sim 90^\circ$ . This contrasts with most other galactic regions, in which the RMs are small at  $|b| \geq 12^\circ$ .

### Other new zones of high RM off the galactic plane

To display the large-scale RM features better, we have calculated the average RM of neighbouring sources within a  $10^\circ$  radius of each source. Sources with RMs greater than  $1.3\sigma$  from the mean are omitted from the averaging calculation. Figure 2 shows the average RM centred on each source. Inspection of Fig. 2 reveals that there are two other zones of systematically large RM above  $|b| = 5^\circ$ ; one (region B), centred near  $l = 255^\circ$ , extends to negative latitudes  $b \sim -20^\circ$ , and has large positive RMs of up to  $300 \text{ rad m}^{-2}$ . A third high-RM zone (region C) at positive latitudes is centred at  $l \sim 40^\circ$ ,  $b \sim +5^\circ$ .

We note that in the south galactic hemisphere the prevailing RM sense as previously noted by Gardner, Morris and

Whiteoak<sup>2</sup>, and Vallée and P.P.K.<sup>5</sup>, is negative in  $0^\circ \leq l \leq 180^\circ$  and positive at  $180^\circ \leq l \leq 360^\circ$ . This trend continues from the galactic plane as far as the south galactic pole, and is reinforced by the present, larger RM sample. The overall trend of RMs in the northern galactic hemisphere (NGH) is less straightforward. At latitudes about  $+20^\circ$  the prevailing RM sense is positive for  $0^\circ \leq l \leq 90^\circ$  (which we denote as the first quadrant), and the sign alternates in successive quadrants in the NGH. One explanation of this pattern, which was also recognised by Gardner *et al.*<sup>2</sup> and Vallée and P.P.K.<sup>5</sup>, is that loop I (the North Galactic Spur, see ref. 10) seems to cause a perturbation of the local magnetic field on a large angular scale in the first and fourth quadrants ( $0^\circ \rightarrow 90^\circ$ , and  $270^\circ \rightarrow 360^\circ$ ) in the NGH. The outer two quadrants reflect the trend of RM present at southern latitudes.

### Rotation measures and the galactic loops

The perturbation of the RMs in the first and fourth quadrants and  $b > +20^\circ$  is consistent with a large magnetic loop associated with loop I which is the largest 'off-plane' feature of the galactic continuum radiation<sup>11</sup>. We can now establish the magnitude of this perturbation as  $\approx 30 \text{ rad m}^{-2}$ . This is much weaker than features A, B and C mentioned above. Feature C, being close to the plane, is probably not associated with loop I.

Loop III (ref. 12) in the northern hemisphere has no corresponding large-scale RM feature greater than  $\sim 20 \text{ rad m}^{-2}$  (Figs 1, 2). The fact that loops I and III do not appear as strong features in the RM sky is consistent with other evidence that they are local features.

Loop II (ref. 12), on the other hand, seems to encircle RM feature A. Whether or not this is merely coincidence is not clear, but we also note that region A coincides with a mild enhancement of the galactic continuum radiation. If loop II is similar in nature to loops I and III and if it is also associated with feature A, the very large RMs in A require it to be too large to be local. A mean RM at  $l = 90^\circ$ ,  $b = -25^\circ$  of  $-150 \text{ rad m}^{-2}$  implies, for  $\langle n_e \rangle = 0.03 \text{ cm}^{-3}$  and an aligned magnetic field of  $3 \times 10^{-6} \text{ G}$ , a scale length of 2 kpc. We conclude that if loop II is a local feature associated with loop III (which is directly on the opposite side of the galactic plane), then it cannot be associated with RM feature A. We consider that loop II is unlikely to be a local feature in view of its close angular coincidence with feature A.

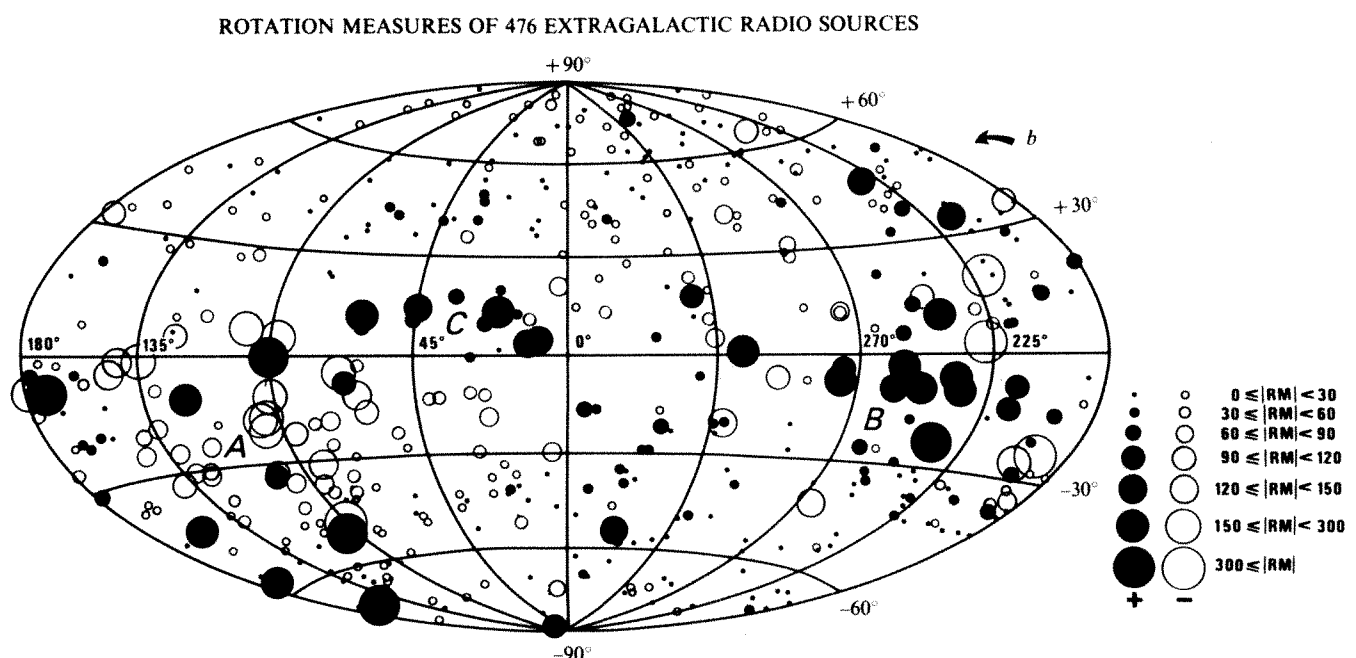
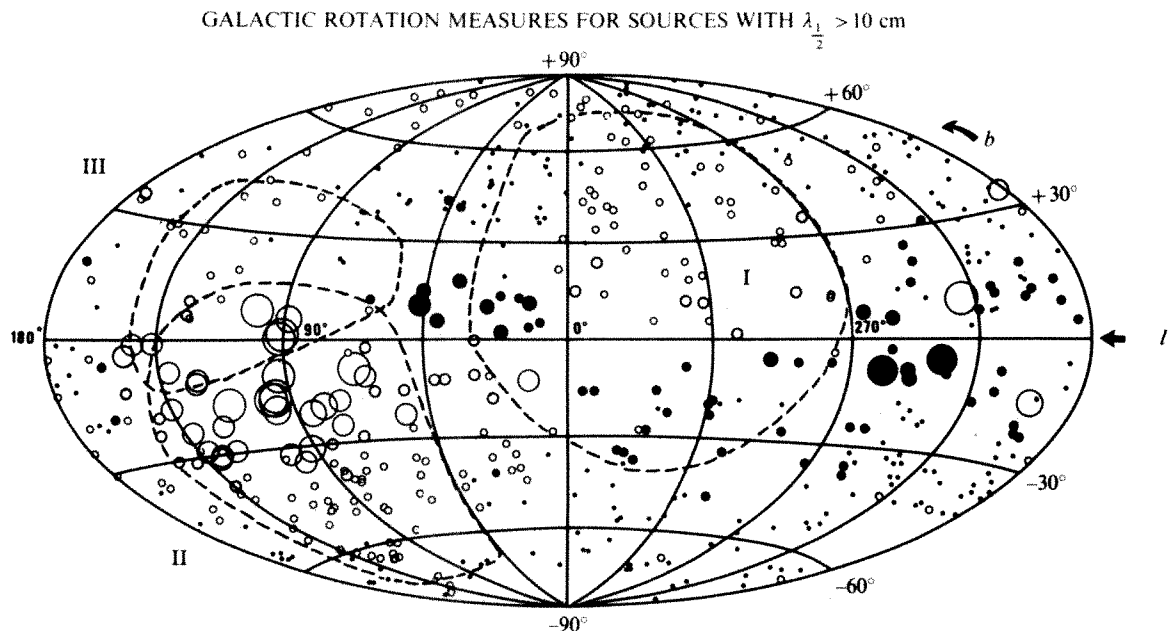


Fig. 1 RMs of 476 extragalactic radio sources displayed on an equal area projection. Features A, B and C (see text) are labelled.



**Fig. 2** Mean RMs in circles of  $10^\circ$  radius centred on each source, which emphasise the large-scale variations better than Fig. 1. The RM scale is the same as that of Fig. 1. The positions of the radio continuum loops I, II and III are outlined. Note that some small-scale features which are real are smoothed out, and the precise location of boundaries where the RM changes sign are slightly masked. These are best located in Fig. 1.

The large scale of feature *A* (implied by its large RM) requires that it have a large magnetic energy,  $\gg 10^{52}$  erg. On this consideration alone, RM feature *A* is too energetic to be associated with a supernova remnant. On energetic grounds it could be associated with either galactic rotation or large-scale streaming of the infalling high-velocity HI clouds, of which there are several in the same direction<sup>13,14</sup>. It is possible that feature *A* is associated with local streaming effects which are related to the magellanic stream, because the end of the magellanic stream lies in the approximate direction of feature *A*. More data are required, however, to establish any connection with the magellanic stream.

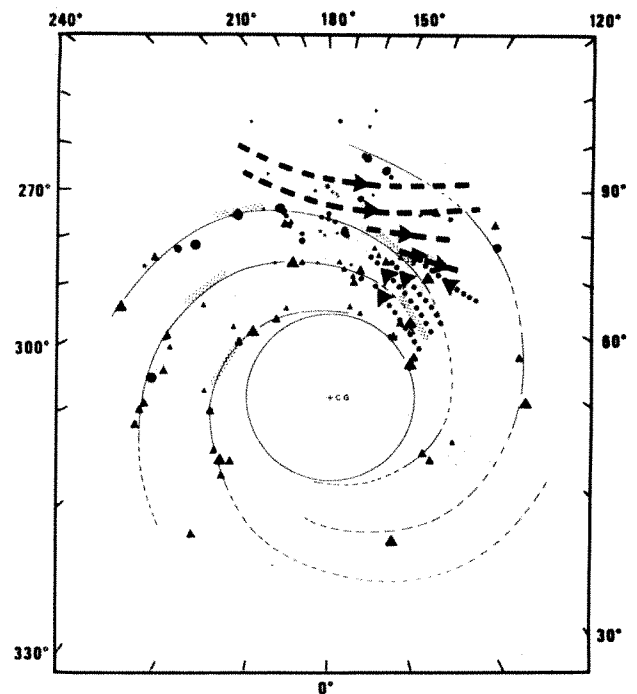
### The structure of magnetic field

The large rotation measures observed near the galactic plane around  $l \approx 255^\circ$  (feature *B*) and  $l \approx 90^\circ$  are consistent with a longitudinal magnetic field directed towards  $l \approx 90^\circ$ . In the direction opposite to galactic rotation the large RM zone is centred near  $255^\circ$  rather than  $270^\circ$  which suggests that the Perseus arm may be opening up in this direction. The RMs and dispersion measures (DM) of pulsars in this direction indicate a mean magnetic field of less than  $3 \mu\text{G}$  ( $90^\circ$  and  $270^\circ$  south of the galactic plane). From this we conclude that the extent of the aligned field in both these areas (*A* and *B*) is greater than  $\sim 4$  kpc. *A* and *B* must therefore be large-scale features of the Galaxy. Feature *C* at  $l \approx 45^\circ$  can be interpreted as another major longitudinal component located approximately between the Sagittarius and the Norma-Scutum arms where (as the magnitude of the RM suggests) the prevailing field is directed along our line of sight for several kiloparsecs.

The very abrupt reversal of RM near the plane at  $l \approx 60^\circ$  is consistent with a model containing two prevailing field zones pointing in opposite directions along the spiral interarms. This is best shown in Fig. 3, in which we superimpose the model of the spiral structure of the Galaxy by Georgelin<sup>15</sup>.

The fields do not seem to be co-planar. They also extend to quite large angular distances from the mean galactic plane. An interesting question at this stage is whether a counterpart to, say, region *C* can be seen on the  $l < 360^\circ$  side of the Galaxy—in the southern hemisphere. Unfortunately, we have not made

measurements from the southern hemisphere, and have calculated RMs only from polarisation measurements in the literature for this region. It is now clearly of interest to obtain more data at  $|b| < 30^\circ$  in the range  $360^\circ \geq l \geq 260^\circ$ . This would verify if large-scale magnetic field components can be seen associated with arm or interarm regions on the southern side of the Milky Way.



**Fig. 3** A sketch of the regions containing an aligned component of magnetic field, indicating their approximate scale and direction. This is shown superimposed on the distribution of bright HII regions in the Galaxy by Georgelin<sup>15</sup>. Note that the heavy dotted and dashed lines do not necessarily represent the local prevailing magnetic field direction, but rather the zones in which the line-of-sight integral of RM is large and has the same sense.



RM of pulsars in the direction  $l = 60^\circ$  near the plane indicate a more turbulent field exhibiting several reversals along the line of sight. Interarm magnetic fields, on the other hand, tend to be more homogeneous, as suggested by the fact that there are virtually no small rotation measures in features *A* and *B*.

## Conclusion

We conclude that galactic loops I and III cause at most weak perturbations in the RM sky. Loop II may or may not be a local loop like loops I and III, depending on whether or not it is

associated with RM feature *A*. The latter must be at least 2 kpc and possibly as large as 6 kpc in length.

Weak fields ( $< 3 \mu\text{G}$ ) seem to occupy the interarm regions and maintain the same prevailing sense for distances of several kiloparsecs. A reversal of the prevailing field direction occurs near  $l = 60^\circ$ . The field direction seems to change abruptly as our line of sight crosses the Sagittarius arm. A more detailed and quantitative analysis of the galactic RM and magnetic structure will be published separately.

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# Preliminary correlations between the Koobi Fora and Shungura Formations, East Africa

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*Major, minor and trace element analyses of glass from pumice and tuffs from the Koobi Fora and Shungura Formations show that the Chari, Karari and Tuff L are correlative; that the KBS Tuff and Tuff H2 are correlative; and that an unnamed tuff in the Koobi Fora Formation is correlative with Tuff H4 of the Shungura Formation.*

TWO important early Pleistocene hominid-bearing formations, the Koobi Fora Formation and the Shungura Formation, are found in the Lake Turkana Basin in northern Kenya and in southern Ethiopia, respectively (Fig. 1). These formations have yielded several hundred hominid specimens<sup>1,2</sup> and several thousand artefacts<sup>3,4</sup>. Despite almost 10 years of work in the region, there are no firm stratigraphic correlations between the two areas. Lack of exposure in the intervening area precludes correlations based solely on field studies.

Rhyolite tuffs, mostly reworked, occur in both regions and have served as marker beds for mapping, and have been useful for K–Ar dating. Over 100 tuffs occur in the Shungura Formation, and 12 of these are extensive enough to subdivide that formation into 12 members. The thick stratigraphic section, good exposure and small lateral facies variations have resulted in a well-established sequence<sup>5,6</sup>. The Koobi Fora Formation was deposited near the basin margin by different, smaller river systems that drained the Suregei Cuesta and the Chew Bahir

(Lake Stephanie) region<sup>7</sup>. Mapping of the Koobi Fora Formation has been hampered by poor exposure and rapid lateral facies changes. Independent dating and palaeontological studies in the two regions led to faunal correlations that, if acceptable, are very disconcerting<sup>8,9</sup>. If the chronologies of both formations were correct<sup>5,10</sup>, faunas of similar evolutionary stage are separated by 0.5–1 Myr. Subsequent dating of pumice from the KBS Tuff (1.8 Myr)<sup>11</sup> in the Koobi Fora Formation did not substantiate the original date of 2.6 Myr<sup>10,12</sup>. This discrepancy has led to additional studies<sup>13,14</sup> which disagree with the younger date.

Volcanic ash is an excellent correlative tool in Quaternary studies. Mineral suites, refractive indices of minerals and glass, and the chemical composition of glass have all been successfully used to correlate air-fall tuffs<sup>15,16</sup>. Reworked ash deposits, however, have additional complications. Erosion of pre-existing ash deposits and addition of pumice and glass from these deposits, abrasion of distinctive glass mantles on primary minerals and dilution of primary minerals by detrital minerals render several techniques useless. Primary minerals in Koobi Fora Formation tuffs are sometimes unrecognisable because of abrasion; electron microprobe studies of individual shards show that some tuffs are made up of several populations of shards with differing chemical compositions<sup>17</sup>. Because differing proportions of these populations would make trace element analyses of bulk glass samples of dubious value, the present study concentrated on characterising pumice fragments from tuffs because electron microprobe studies showed that the glass in a single pumice is homogeneous.

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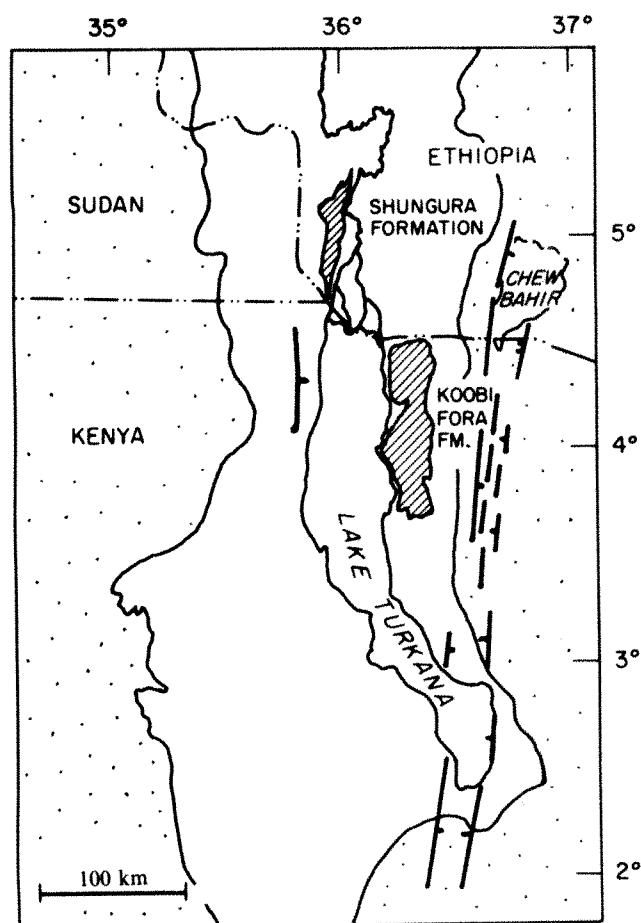


Fig. 1 Map of Koobi Fora and Shungura Formations.

## Procedure

Tuff and pumice samples were collected during several field seasons (1972–77). Pure glass separates from individual pumice were obtained by gentle crushing and sieving, treatments with HCl and HF in an ultrasonic bath to remove adhering clay and silt particles, and repeated magnetic separations. Heavy liquids were used only as a last resort to keep bromine contamination to a minimum. Glass was mixed with cellulose and pressed into pellets for X-ray fluorescence analysis. USGS standards G-2 and GSP-1 were used, and results were corrected for absorption. Glass shards from tuff and pumice samples were analysed by electron microprobe for seven elements and were corrected for matrix effects<sup>18</sup>.

## Results: tuff correlations and ages

Three tuffs stand out as having identical chemical properties and are thus considered to be the same tuff. Figure 2 summarises the chemical data for the Chari, Karari and KBS Tuffs from the Koobi Fora Formation and Tuffs H2, H4 and L from the Shungura Formation.

**Chari–Karari–L:** the Chari and Karari tuffs of the Koobi Fora Formation cap their respective sections at Ileret and along the Karari Ridge. These two tuffs have been thought to be correlative on the basis of stratigraphic position<sup>7</sup>, K–Ar dates<sup>10</sup> and similar faunas in the underlying strata<sup>9</sup>. Trace and minor element contents of glass from pumice confirm this presumed correlation (Fig. 2). Electron microprobe analyses of individual shards from tuffs and of glass from pumice show that both have the same composition. One pumice cobble from Tuff L of the Shungura Formation has the same major, minor and trace element composition as pumice from the Chari and Karari Tuffs (Fig. 2, Tables 1, 2). A glass separate from Tuff L is nearly identical in trace element chemistry to that of the pumice (A. Martz, personal communication). Feldspars from pumice in the

Chari and Karari Tuffs and from Tuff L range in composition from about  $An_{10}Ab_{28}Or_{12}$  to  $Ab_{64}Or_{36}$ . Average calcium, iron and barium contents in these feldspars over the compositional range  $Or_{32}–Or_{36}$  are given in Table 3. The compositional range over which the average was taken is restricted because both barium and iron vary systematically with composition of the feldspar, and also to ensure comparability between different data sets on feldspars with different compositional ranges. K–Ar dating on nine feldspar separates range from 1.26 to 1.48 Myr and average about 1.35 Myr (R.E.D., in preparation). This agrees with previous results<sup>10</sup>.

As these tuffs have identical ages (R.E.D., in preparation) and chemical compositions, and because their feldspars are nearly identical in composition, they are probably products of the same eruption, and represent the same time horizon even though they were deposited by different drainage systems. This may indicate that the source area is on the drainage divide separating the two areas, as postulated earlier<sup>19</sup>.

Present nomenclature is confusing; the name 'Karari Tuff' should be eliminated as the name 'Chari Tuff' has precedence. Because both the terms 'Chari Tuff' and 'Tuff L' are firmly entrenched in the literature, however, we propose to use each term in its respective section.

**KBS-H2:** nine pumice fragments from the KBS Tuff in area 131, two pumice fragments from near the KBS type locality in area 105, and two pumice fragments from Tuff H2 were analysed (Fig. 2). Three distinctive pumice types are found in area 131: very dark brown glass with large vesicles containing about 5.5%  $Fe_2O_3$  (one sample); grey glass with moderate vesicles having about 4.2%  $Fe_2O_3$  (three samples); and silky white glass with stretched tubules having about 3.1%  $Fe_2O_3$  (five samples). This tuff consists of platy and stretched glass

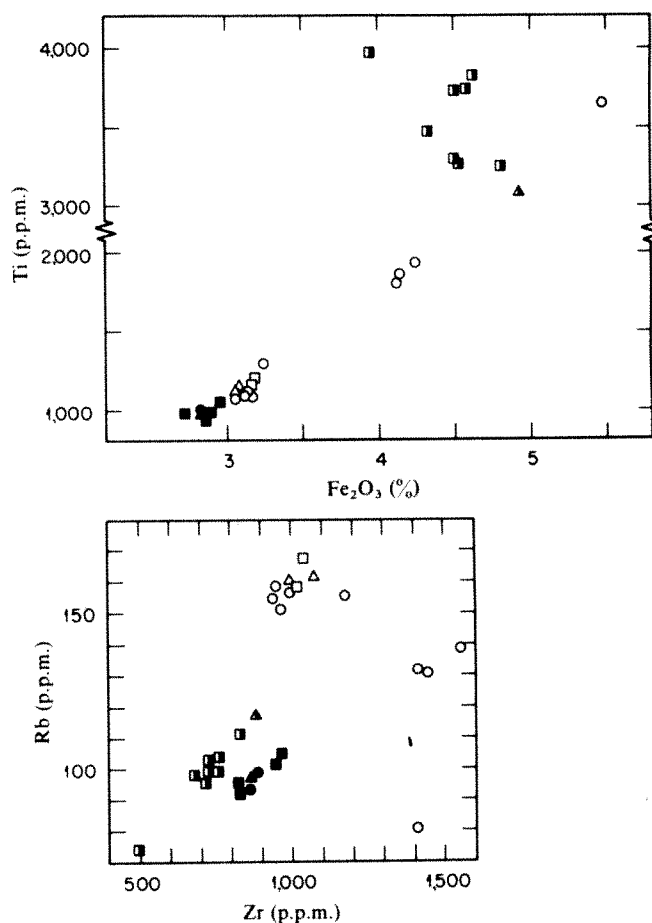


Fig. 2 Trace and minor element contents of pure glass separates from pumice from tuffs in the Koobi Fora and Shungura Formations. ●, Chari Tuff; ■, Karari Tuff; ▲, Tuff L; ■, KBS Tuff-105-East; ▴, Tuff H4; ○, KBS-131; □, KBS-105; △, Tuff H2.

**Table 1** Major and minor element compositions of glass from tuff and pumice

Unit	Material	Area	Sample no.	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	CaO	MgO	Fe <sub>2</sub> O <sub>3</sub>	TiO <sub>2</sub>	MnO
Chari	Tuff	6	77-23	74.7	10.7	0.18	0.02	2.82	0.16	0.06
Chari	Pumice	6	6-001	75.8	10.5	0.17	0.02	2.76	0.16	0.05
Karari	Tuff	131	77-19	74.5	10.6	0.19	0.03	2.86	0.19	0.06
Karari	Pumice	118	108-001	75.4	10.5	0.19	0.02	2.86	0.17	0.05
Tuff L*	Pumice	Omo	76-L	74.6	10.5	0.19	<0.05	2.82	0.17	0.07
KBS	Tuff	102	72-44	73.9	10.9	0.19	0.05	3.07	0.18	0.11
KBS	Tuff	105	77-15	72.9	10.8	0.17	0.04	3.06	0.18	0.08
KBS	Tuff	131	75-2t	74.8	10.9	0.18	0.06	3.08	0.18	0.09
KBS	Pumice	131	75-2E	74.2	10.9	0.20	0.06	3.01	0.22	0.09
KBS	Pumice	131	75-2F	73.1	10.9	0.21	0.07	3.22	0.20	0.09
H2	Pumice	Omo	76-H2A	73.1	10.9	0.18	0.05	3.16	0.19	0.10
H2	Pumice	Omo	76-H2B	73.4	10.9	0.19	0.05	3.14	0.19	0.10
KBS	Pumice	131	75-2C	70.4	11.2	0.35	0.07	4.26	0.32	0.15
KBS	Pumice	131	75-2G	72.3	11.2	0.34	0.07	4.29	0.33	0.14
KBS	Pumice	131	131-002	72.0	10.9	0.34	0.08	4.38	0.32	0.14
KBS	Pumice	131	131-001	68.8	12.7	0.77	0.27	5.70	0.58	0.34
KBS*	Pumice	105-E	77-50A	70.6	10.6	0.34	0.3	4.80	0.54	0.24
H4*	Pumice	Omo	76-H4B	71.9	10.8	0.35	0.2	4.92	0.51	0.23

Determinations are by electron microprobe unless otherwise specified. Total iron as Fe<sub>2</sub>O<sub>3</sub>.

\*Analysis by X-ray fluorescence.

shards; generally about 30% of these are brown to dark brown and close examination shows that there is a continuum from the colourless to the dark shards. Locally, basal portions of the tuff lack the dark shards. Electron microprobe studies show no difference in chemical composition between light and dark shards and indicate that they both have the same composition as the most common pumice type (that with about 3.1% Fe<sub>2</sub>O<sub>3</sub>, see Table 1). The three types are easily distinguished on the basis of their trace element chemistry (Table 2, Fig. 2). K-Ar dates were determined on all three types of pumice to determine whether older pumice had been reworked into a younger tuff. If this is the case, the pumices involved must differ in age by less than the analytical error (about  $\pm 0.03$  Myr), as the groups had average

dates of 1.82, 1.83, and 1.84 Myr, respectively (R.E.D., in preparation). Concordant glass-feldspar pairs were found for two pumice (R.E.D., in preparation).

Two pumice from the type KBS Tuff in area 105 have the same composition as the dominant group in area 131; electron microprobe analyses on glass shards from the tuff show that it is the same tuff as in area 131 (Table 1). A conventional K-Ar date of 1.75 Myr was obtained for one feldspar separate (R.E.D., in preparation). Two pumices from Tuff H2 in the Shungura Formation also have the same composition as the dominant pumice type in area 131 (Fig. 2). Electron microprobe analyses of glass from the pumice confirm this conclusion (Table 1). Four conventional K-Ar analyses (three from samples not analysed

**Table 2** Results of X-ray fluorescence analyses on glass separates from pumice fragments

Tuff	Area	Sample no.	Nb	Zr	Y	Sr	Rb	Zn	Mn	Ti	Fe <sub>2</sub> O <sub>3</sub>
Chari	6	6-001A	106	885	84	4	109	178	579	1000	2.82
Chari	1	1-9902CC	103	864	82	1	105	179	588	992	2.84
Karari	131	108-1	104	844	82	40	105	176	599	952	2.85
Karari	131	108-2	114	940	89	36	111	189	617	1059	2.95
Karari	131	108-3	107	832	81	9	103	178	573	980	2.71
Karari	131	108-4	118	964	92	5	115	183	595	996	2.88
Tuff L	Omo	76-L	105	867	90	7	107	176	576	999	2.82
KBS	131	75-2A	224	972	105	—	162	218	826	1129	3.13
KBS	131	75-2B	221	946	105	—	165	213	832	1080	3.05
KBS	131	75-2D	225	968	109	—	169	221	854	1098	3.16
KBS	131	75-2E	198	1002	115	11	168	219	841	1130	3.13
KBS	131	75-2F	213	1176	117	6	166	238	933	1305	3.24
KBS	105	77-108A	201	1016	113	4	169	220	836	1170	3.16
KBS	105	77-108B	219	1057	117	1	177	226	853	1203	3.17
H2	Omo	76-H2A	218	1014	110	4	170	220	835	1120	3.05
H2	Omo	76-H2B	201	1083	113	7	172	221	833	1146	3.06
KBS	131	75-2C	254	1412	109	—	142	252	1309	1811	4.11
KBS	131	75-2G	231	1436	110	5	141	254	1287	1889	4.14
KBS	131	131-002	239	1553	116	2	149	260	1373	1953	4.24
KBS	131	131-001	176	1408	76	5	91	198	2786	3611	5.48
KBS	105-E	77-109B	104	492	49	5	73	132	1615	3994	3.94
KBS	105-E	LUCAS-C	130	686	63	8	98	164	1762	3475	4.32
KBS	105-E	LUCAS-B	132	707	68	8	98	172	1877	3753	4.52
KBS	105-E	LUCAS-A	135	727	69	8	100	170	1847	3744	4.57
KBS	105-E	77-109D	154	741	67	15	103	154	1705	3293	4.51
KBS	105-E	77-109A	142	757	71	10	103	159	1767	3288	4.53
KBS	105-E	105-004	149	734	67	2	99	166	1882	3823	4.61
KBS	105-E	77-50A	171	830	74	19	111	189	1799	3245	4.80
H4	Omo	76-H4B	176	876	79	16	117	201	1822	3081	4.92

In p.p.m. except total iron, which is reported as % Fe<sub>2</sub>O<sub>3</sub>.



**Table 3** Minor element content of feldspars

Sample no.	KA no.	Tuff	Area	CaO	BaO	Fe <sub>2</sub> O <sub>3</sub> *
108-1	2815	Karari	118	0.08	0.25	0.41
6-001A	2865	Chari	6	0.11	0.26	0.41
108-4	2880	Karari	118	0.09	0.31	0.36
108-2	2882	Karari	118	0.09	0.31	0.34
76-L	3254	Tuff L	Omo	0.10	0.29	0.36
75-2A	3160	KBS	131	0.08	0.07	0.43
75-2D	3161	KBS	131	0.06	0.06	0.43
77-108A	3189	KBS	105	0.08	0.05	0.38
76-H2A	3256	H2	Omo	0.08	0.06	0.42
75-2C	3166	KBS	131	0.09	0.06	0.36
75-2G	3167	KBS	131	0.08	0.02	0.45
131-002	2842	KBS	131	0.07	0.05	0.47
131-001	2869	KBS	131	0.28	0.11	0.38
77-50A	3182	KBS	105-E	0.14	0.03	0.65
	—	H4	Omo	0.10	0.04	0.69†

Averages taken over the range Or<sub>32</sub>–Or<sub>37</sub>.\*Total iron expressed as Fe<sub>2</sub>O<sub>3</sub>.

†Analysis taken from ref. 5.

by X-ray fluorescence and from an earlier study<sup>5</sup>) average 1.87 Myr (R.E.D., in preparation).

There are several types of feldspars from the KBS Tuff. Those from pumice samples, where the iron content of the glass is about 3.1 or 4.2%, are indistinguishable chemically, and range from about An<sub>8</sub>Ab<sub>76</sub>Or<sub>16</sub> to Ab<sub>62</sub>Or<sub>38</sub>. Those from samples, where the iron content of the glass is about 5.5%, range from about An<sub>16</sub>Ab<sub>73</sub>Or<sub>10</sub> to Ab<sub>63</sub>Or<sub>37</sub> and are distinctly higher in calcium and barium. Averages for all points with greater than 32% Or are given in Table 3. These feldspars are clearly distinct from the feldspars of the Chari-L Tuff. A third feldspar type is found in the KBS Tuff from area 105-E; it is distinct from the other two and is discussed below.

## Discussion

The combined evidence justifies a correlation between the KBS Tuff of the Koobi Fora Formation in areas 131 and 105 and Tuff H2 of the Shungura Formation because of the close correspondence in age (R.E.D., in preparation) and composition. The age of 1.8 Myr for this tuff (R.E.D., in preparation) is compatible with palaeomagnetic studies<sup>20</sup>, which place this tuff in a normally polarised section, and with faunal evidence<sup>9</sup>. We propose that this ash should continue to be called by its original term to avoid confusion in future studies.

KBS (area 105-East)-H4: the tuff that occurs in area 105-East is not the KBS Tuff because no pumice characteristic of that found in the KBS Tuff in areas 105 and 131 are found in this area. Compositions of glasses from pumice show that they are members of an alkaline-rhyolite differentiation series (Fig. 2, Table 2). A single pumice lump from Tuff H4 of the Shungura

Formation also falls on this differentiation trend. Composition of minor element chemistry of feldspars from pumice also show them to be related (Table 3, samples 77-50A and H4). Conventional K–Ar dates on these samples average 1.8 Myr. Although this tuff is similar in age to the KBS Tuff and Tuff H2, and also occurs in normally polarised sediments, stratigraphic relationships in the Shungura Formation show that Tuff H4 occurs about 15 m above Tuff H2<sup>21</sup>; this tuff is therefore younger than Tuff H2. Because these pumice from the KBS Tuff in area 105-East and Tuff H4 have indistinguishable chemistries, feldspar compositions, and conventional K–Ar dates (R.E.D., in preparation) they are probably the same tuff.

Correlation of the Chari and Karari Tuffs with Tuff L, of the type KBS Tuff with Tuff H2, and of the tuff in area 105-East with Tuff H4 of the Shungura Formation is independent of the controversy surrounding the age of the KBS Tuff<sup>9–14</sup>. However, the correlations presented here are compatible with palaeomagnetic studies<sup>20,21</sup> and additional K–Ar dates on other tuffs within the Koobi Fora, Kubi Algi and Shungura Formations<sup>5</sup>.

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# Application of 'molecular' theories to the structure of the crystalline state

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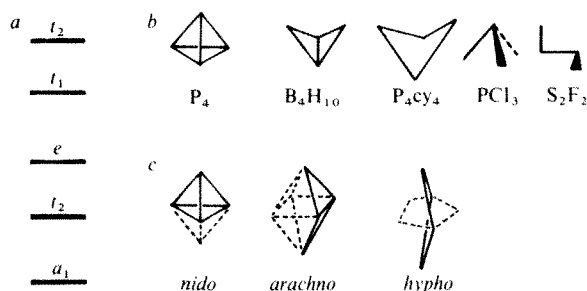
*Recent years have seen the development of simple molecular orbital based ideas to view the structures of molecular ring, cage and cluster compounds. The principles of these methods are used here to demonstrate in a new way how the structures of solids depend on electronic configuration.*

IN contrast to the structures of simple molecules there are many possibilities for the geometrical arrangement of simple systems as extended arrays in solids. We may pinpoint three general factors which determine a solid state structure. First, the efficient packing together of the atoms in the solid. The occurrence of a particular AX structure will often be restricted if the radius ratio

of the two atomic species A and X is unfavourable. Second, the preservation of long-range order. Many structures look very favourable when a small cluster of atoms is built up but sometimes completion of another coordination shell is not possible whilst retaining the symmetry of the structure. Third, electronic forces. These may be divided into ionic, non-directional effects and covalent forces, directional in character. This article shows how recent developments in understanding the structures of molecular ring, cage, and cluster species may be used to provide a new way of viewing the structures of solids.

### Theories for molecular species

One way, due to Mingos<sup>1</sup>, that we can look at the geometries of these cages and rings is to start by taking the molecular orbital structure of a simple symmetric system and inquire what happens to the number of skeletal bonds as we add electrons to the system. Figure 1 shows a molecular orbital diagram for a tetrahedral  $A_4$  species. It is characterised by the fact that there are six low energy orbitals holding the framework together and to higher energy six orbitals which are antibonding between the A atoms. The four orbitals which point outwards from the tetrahedron and do not take part in framework bonding are not shown. With five electrons per A atom there are just enough to fill all the six skeletal bonding orbitals (number of skeletal electron pairs,  $n = 6$ ) and the outward pointing orbitals (lone pairs). This is the case for the electron precise tetrahedral molecule  $P_4$  (Fig. 1) where there are six P-P linkages. For  $n = 7$  two electrons must go into an antibonding orbital and one of the AA bonds is broken. Thus the structure found for  $B_4H_{10}$  is an opened-out tetrahedron. With  $n = 8$  a similar structure results for  $P_4(cy)_4$  (cy = cyclohexyl) but with no central linkage. For  $n = 9$  three of the tetrahedral bonds must be broken. This is a novel way of looking at  $PCl_3$  or  $S_2F_2$ . The presence of a central atom does not disturb this simple picture (but we must be careful to count the number of skeletal electrons properly) and with 12 'skeletal' pairs  $CCl_4$  has no bonds between the A (in this case Cl) atoms. An analogous scheme holds for the rationalisation of the structures of many transition metal cluster complexes which are difficult to understand in more conventional ways.

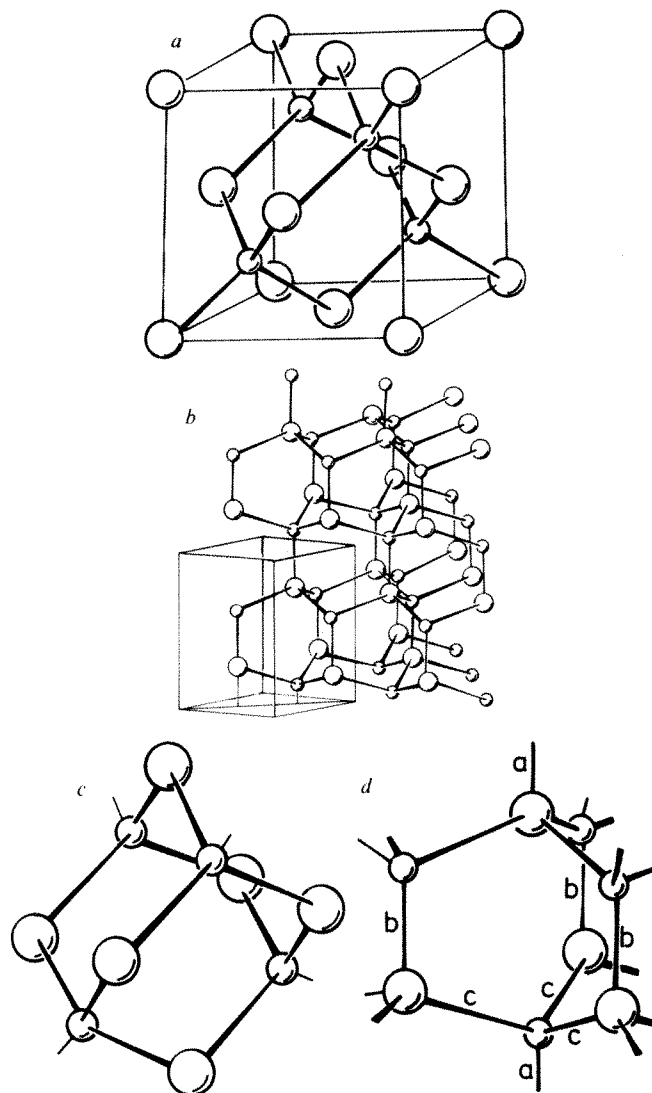


**Fig. 1** a, Schematic molecular orbital picture of a tetrahedral  $A_4$  species; b, the structures of some four atom units; c, the latter viewed using Wade's scheme.

The second approach, due to Wade<sup>2,3</sup>, is again based on the number of skeletal electrons in the molecule,  $n$ . The resultant structure is based on that of an  $N$  vertex deltahedron where  $N = n - 1$ . If the carborane or cage compound has  $N$  skeletal atoms then all the vertices will be occupied (a *closo* structure) if  $N - 1$  atoms all the vertices but one will be occupied (a *nido* structure), if  $N - 2$  atoms then all the vertices but two will be occupied (an *arachno* structure), if  $N - 3$  atoms then all the vertices but three will be occupied (a *hypho* structure), and so on. Figure 1 also shows how the  $P_4$ ,  $B_4H_{10}$  and  $P_4cy_4$  molecules are viewed on this scheme, as *nido*, *arachno* and *hypho* species. Various rules<sup>4</sup> have been developed to predict which vertices will be left vacant in *nido*, *arachno* and *hypho* structures.

### Bond breaking and the number of valence electrons

A large number of AX systems with eight valence electrons per formula unit adopt the zincblende (sphalerite) or wurtzite structure (Fig. 2.) ZnS, carbon (diamond), AlSb, CuCl, CuBr, HgS are among the systems which adopt the former structure and



**Fig. 2** a, The zincblende (sphalerite) and b, wurtzite structures; c, part of the zincblende structure indicating a 'unit cage'; d, unit cage of the wurtzite structure (other ways of choosing the cage are, of course, possible).

ZnO, AlN, BeO, CdS, CuI, MgTe the latter. Some of the examples are dimorphic. (For details of these and other systems cited here see refs 5-7.) Extraction of a chunk of lattice from each structure reveals the basic building block and demonstrates the similarity between the two systems. We recognise in these two lattice fragments the framework found for adamantane or  $P_4O_6$  and  $P_4O_{10}$  (zincblende) or bicyclo(222)octane or barrelene (wurtzite) in molecular systems. How the molecule (or solid) is held together is best illustrated for the case of the diamond configuration. Each carbon atom has four, equivalent, tetrahedrally directed orbitals on a localised equivalent molecular orbital picture, made by suitable combination of 2s and 2p orbitals. With four electrons, each carbon atom contributes half a bonding electron pair to either a cage bond (that is towards the framework of the structures of Fig. 2c, d) or to an outward pointing bond which connects this cage to another. Thus no electrons go into antibonding orbitals and there are the right

number of electrons to make four bonds around each carbon atom. In the case of molecular  $P_4O_6$  the outward pointing orbitals are filled with lone pairs of electrons leaving just 24 electrons, (that is 12 pairs) to make all 12 intracage bonds. These are examples of electron precise structures and regular geometries are found. But what happens when more than eight electrons per atom pair are present? According to the approach above, bonds are broken. Equation 1 shows one series of structures we will follow which show that the 'molecular' approach also works well for solids.

Wurtzite:	$ZnS \rightarrow \beta GaSe \rightarrow As \rightarrow Se, Te \rightarrow I_2 \rightarrow Xe$	1
No. of electrons:	8    9    10    12    14    16	

We start by considering the wurtzite structure. Each cage (Fig. 2d) contains two formula units. The three AX units forming the sides of the prism are shared by three such cages, the 'apical' AX unit is associated with only one cage. The apical atoms are attached to another cage by a single linkage top and bottom. The prismatic atoms are each attached to two other cages. There are thus 16 electrons per cage, all residing in bonding orbitals. With one extra electron per formula unit (for example GaSe) there are sufficient electrons to break one two-electron-bond per cage. We need to count up the number and type of the bonds present in the cage very carefully in order to see how many bonds may be broken. We may break either two extra-cage linkages, (a) in Fig. 2d, three prismatic cage linkages (b) or two pyramidal (c) linkages with these electrons. If extra-cage bonds are broken

then each cage will contribute to the bond breaking, and two of these bonds may be broken for each pair of electrons contributed by a single cage. If cage bonds themselves are broken, the number which may be broken per extra electron pair per cage depends on the number of cages that share a particular linkage: three for the prismatic bonds, b, and two for the pyramidal bonds, c. The structure of  $\beta$ -GaSe (Fig. 3a) shows that the bonds broken are either both the a bonds or all three b bonds, the result being indistinguishable because of the symmetry of the structure. It is important that the number and type of linkages broken compared to the wurtzite structure accords with the number of extra electrons present. Two other differences are of note by comparison with the wurtzite geometry. First, after bond fission, each of the layers moves with respect to the one beneath it. This clearly aids the packing together of the layers. Second, a readjustment of the atom positions within the cage has occurred. The Se atoms only occupy the apical positions of the wurtzite cage fragment. GaTe has a related structure with the Te atoms in the apical sites but with some other differences.

A structure where different linkages of the wurtzite cage are broken is that of wolfsbergite  $CuSbS_2$ . If we write this as  $Cu(I)Sb(III)S_2$  to satisfy divalent sulphur then the copper contributes one 4s electron to the electron count. Antimony contributes five electrons and this leads on average to nine electrons per AS unit that is one more than in  $ZnS$  and makes this structure iso-electronic with GaSe. Wolfsbergite is a superstructure of wurtzite and we can readily see from Fig. 3b that two pyramidal SbS linkages are broken.

Again the sites of lowest coordination, where the breakage has occurred contain the most electronegative species. This observation accords with a general feature of molecular cage compounds. The sites of lowest coordination are invariably those carrying the highest charge. Just as in discussions<sup>8</sup> of the forces determining ligand site preferences in molecular compounds the most electronegative ligand will prefer the sites of highest electron density. We do not know at present why wolfsbergite and  $\beta$ -GaSe have different structures. In enargite  $Cu_3AsS_4$  ( $Cu(I)Cu_2(II)As(III)S_4$ ) there is half an electron on average per AS formula unit. This is enough to break only one extra cage bond of the wurtzite unit but is insufficient to produce a separation between two halves of the structure as in  $CuSbS_2$  or GaSe (we need to break two bonds at least) and the result is a distorted structure based on wurtzite, in which the atoms are displaced from their ideal positions. Instead of regular tetrahedral coordination a range of Cu-S and As-S bond lengths are found. If the structure is regarded as being made up of  $Cu_3(I)As(V)S_4$  then the structure is an electron precise one (four electrons on average per AS formula unit) and an undistorted tetrahedral superstructure is expected.

With 10 electrons per formula unit there are now sufficient extra electrons to break two bonds per cage. In addition to the two extra-cage linkages broken in GaSe, the three vertical linkages, b, may also be broken. (Note that each vertical cage linkage is shared by three cages, so that one extra pair of electrons contributed per cage is sufficient for fission of all three.) The As structure is just this and again the layers are moved with respect to each other to aid in packing (Fig. 4a). With 12 electrons one more bond may be broken per cage and the result is the chain structure (Fig. 4b) found for elemental Se or Te. With 14 electrons another bond may be broken and the result is a lattice composed of molecular  $I_2$  units, still retaining (Fig. 5) in fact a geometrical resemblance by packing of the diatomic units, to the parent structures. The almost trivial case of Xe with 16 electrons has no bonds between the atoms and a van der Waals solid results.

Another of the systems that we could have considered (which we will examine in more detail elsewhere) starts with the eight electron rocksalt (NaCl) structure and observes the gradual breaking of bonds as more electrons are added. Thus the layer structures of arsenic and black phosphorus with two extra electrons built from three coordinate atoms are neatly viewed as arising through two different bond-breaking patterns of the

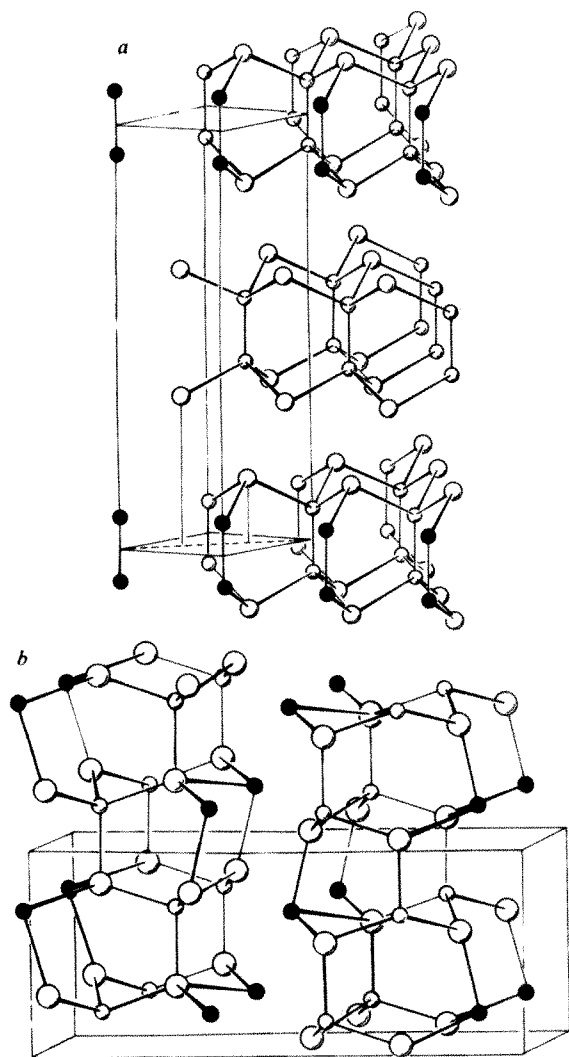


Fig. 3 a, The  $\beta$ -GaSe structure; b, the wolfsbergite ( $CuSbS_2$ ) structure. Large circles Se, small circles Ga or Cu, and small full circles, Sb.

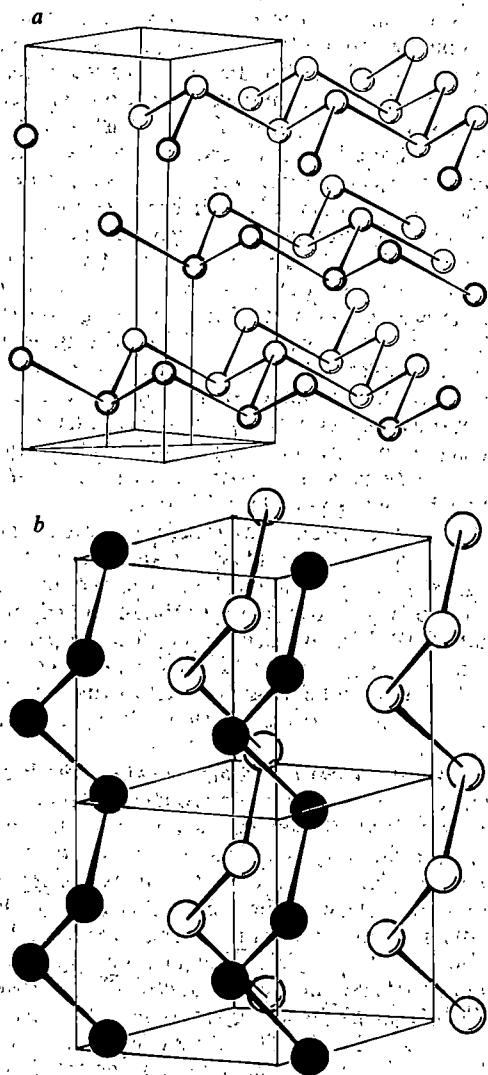


Fig. 4 a, The As layer structure; b, the chain Se, Te structure.

rocksalt structure. TlF and InCl also with two extra electrons have distorted rocksalt-structures where in the latter for example the chlorine atoms are coordinated by three In atoms at 2.91 Å and three at 3.51 Å. InS, with only one extra electron has a structure halfway between that of NaCl and black phosphorus, is a geometry difficult perhaps to understand any other way. Complex sulphur-containing minerals with a non-integral number of electrons per formula unit are also approachable using our scheme. Thus marrite  $\text{PbAgAsS}_3$  contains on average 9.33 electrons per AS atom pair. The structure of this mineral is a distorted superstructure of rocksalt containing six coordinate lead, three coordinate arsenic and three different sulphur environments. Two are four coordinate and one is five coordinate. On average then per atom pair  $1\frac{2}{3}$  'bonds' of the rocksalt structure are broken; close to what would be predicted by interpolation between the results for eight and 10 electron structures. These ideas could be useful as a way to rationalise complex sulphide and sulphosalt structures in general. A similar analysis applies to silicate structures.  $\text{SiO}_2$  itself exists in several modifications, the  $\beta$ -cristobalite and tridymite arrangements being closely related (via an oxygen atom spacer) to those of zincblende and wurtzite. It is also found, with  $\text{Al}^{3+}$  substituted for Si and a cation present to maintain electrical neutrality, as feldspars and zeolites which are also three dimensional networks. ( $\text{SiO}_2$  has 5.33 electrons per atom.) With 5.71 electrons per atom ( $(\text{Si}_2\text{O}_5)^{2n-}$  units are found which give rise to double chains (such as gillespite) and sheet structures (such as micas). With 5.87 electrons per atom ( $(\text{Si}_4\text{O}_{11})^{6n-}$  is reached, and

double chains (such as amphiboles) are found. With 6.00 electrons, the structure is broken up further ( $(\text{SiO}_3)^{2n-}$ ) and linear or cyclic single chains are found as in benitoite and the pyroxenes respectively. With 6.4 electrons per atom isolated  $\text{SiO}_4^{4-}$  tetrahedra are found as in the orthosilicates such as the olivines. This neatly shows that the electron rich species are made up of increasingly smaller units.

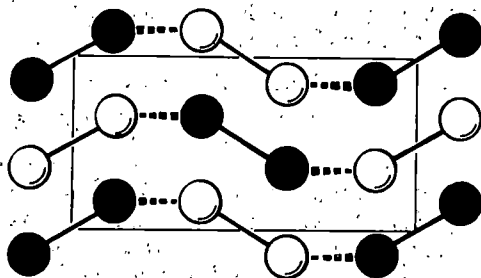


Fig. 5 The  $\text{I}_2$  unit cell. The dashed lines show the similarity to the Se, Te spirals of Fig. 4b.

Extra electrons may be introduced in other ways such as inclusion of an alkali or alkaline earth metal into the structure. Thus  $\text{CaSi}_2$  (10 electrons per  $\text{Si}_2$  unit) has a rumpled sheet structure just like As, and  $\text{CaSi}$  (12 electrons per  $\text{Si}_2$  unit) has zigzag chains just like Se or Te.

Thus, whereas it has been known for a long time that iso-electronic species often have very similar crystal structures we have shown here how the structure adopted may be simply derived. It would be an important step in the classification of the crystalline state if we could relate in general the structure of a particular solid to a reference geometry by consideration of its electronic configuration. We anticipate that this would only work in those systems where covalent or directional forces are important.

### Electron counting schemes

A survey of solid state structures based on the zincblende ZnS structure immediately shows some similarities to Wade's molecular scheme. The  $\text{CdIn}_2\text{Se}_4$  and  $\text{CdAl}_2\text{S}_4$  species crystallise as defect zincblende structures. (Fig. 6). We may represent them as  $\text{Cd}\square\text{In}_2\text{Se}_4$  and  $\text{Cd}\square\text{Al}_2\text{S}_4$  where  $\square$  = vacancy to emphasise their stoichiometric relationship to ZnS. On average per AS or ASe unit ( $A = \text{Cd}, \text{In}, \text{Al}$  or  $\square$ ) there are eight electrons, just as in ZnS and an average of 12 electrons per 'zincblende' cage. This cage structure is neatly regarded as a *nido* ZnS structure. The zincblende structure of Fig. 2 would, of course, be regarded as a *clos*o structure. Examination of a model of this system shows that there are three different zincblende 'cages'. One of these has no atoms missing, the second has two and the third has one.

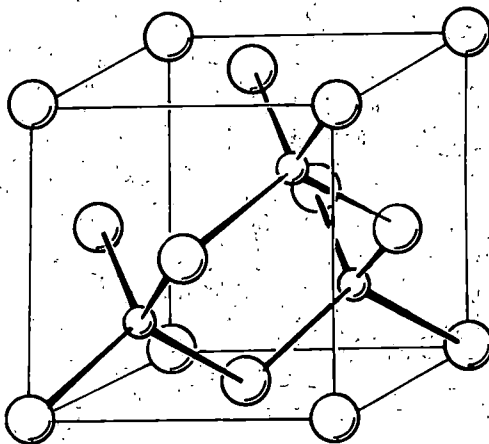


Fig. 6 The defect zincblende structure of  $\text{CdIn}_2\text{Se}_4$ . Large circles Se.



So the structure contains, *closo*, *nido* and *arachno* cages. There are many other examples.

In fact solid state chemists have used electron counting ideas similar to these for many years, which we may formulate as four principles.

(1) Starting with a close packed cubic array of 'anions', filling of the various holes gives the following structures: zincblende (one tetrahedral hole),  $\text{CaF}_2$  (two tetrahedral holes),  $\text{NaCl}$  (one octahedral hole) and so on.

(2) By substituting different cations with the same or average number of electrons in an ordered fashion a superstructure may be built up.

(3) By substituting cations with more electrons per atom some of the sites of the structure remain vacant in an ordered fashion.

(4) By substituting cations with less electrons per atom filled up derivatives are obtained.

Rule (1) is the solid state analogue of the description of the deltahedra on which the molecular structures are based.

Rule (2) has no molecular analogue in the strictest sense as superstructures are essentially a solid state concept but the best

analogy is that similar structures with the same number of skeletal atoms are obtained on substitution of, for example, a BH group by an  $\text{Fe}(\text{CO})_3$  group.

Rule (3) immediately recalls the occurrence of *closo*, *nido* and *arachno* molecular species.

Rule (4) has its molecular counterpart in cluster compounds with a central atom for example,  $\text{Ru}_6(\text{CO})_{17}\text{C}$ . The analogy between the two schemes applied to molecular species and extended arrays is then very striking.

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# The ovalbumin gene region: common features in the organisation of three genes expressed in chicken oviduct under hormonal control

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*Two large DNA fragments overlapping the chicken ovalbumin gene have been isolated by molecular cloning. Analysis of these fragments provided a map of a 46,000-base pair region of the chicken genome. This region contains the complete ovalbumin gene (including its mRNA leader-coding sequence) and at least two other genes of unknown function. All three genes are orientated in the same direction and their expression in chicken oviduct is under hormonal control. The three genes share some sequence homologies, suggesting that duplications have occurred in the ovalbumin gene region in the course of evolution.*

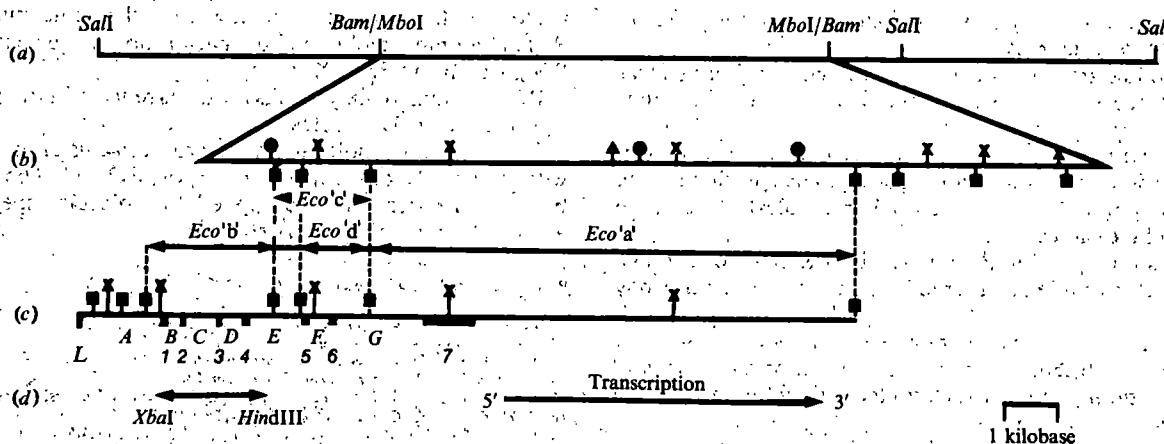
IN the chicken oviduct, the transcription of several genes, including ovalbumin, is strongly stimulated by steroid hormones<sup>1–4</sup>. Knowledge of the structure of these genes should help in the understanding of hormonal control and gene regulation in higher organisms. Following the discovery that the ovalbumin gene is split<sup>5,6</sup> we<sup>7–11</sup> and others<sup>12–14</sup> have analysed its organisation using various methods, including molecular cloning of chicken DNA fragments, and demonstrated the presence of seven introns (intervening sequences) in the ovalbumin gene (Fig. 1c; see ref. 15 for review). Evidence for a 47-nucleotide-long leader sequence at the 5' extremity of ovalbumin mRNA (ov-mRNA) has been obtained<sup>10,14,16</sup>. The leader-coding sequence is not present in any of the gene fragments which we had cloned previously<sup>10</sup>.

In an attempt to isolate the ov-mRNA leader-coding sequence and to characterise the surroundings of the ovalbumin gene, we decided to clone large DNA fragments overlapping the gene. We report here the isolation of two DNA fragments which cover a 46-kilobase region of the chicken genome. This region contains, in addition to the complete ovalbumin gene including its leader-coding sequence, two other genes which are both expressed in the oviduct under hormonal control, and which share some sequence homologies with the ovalbumin gene.

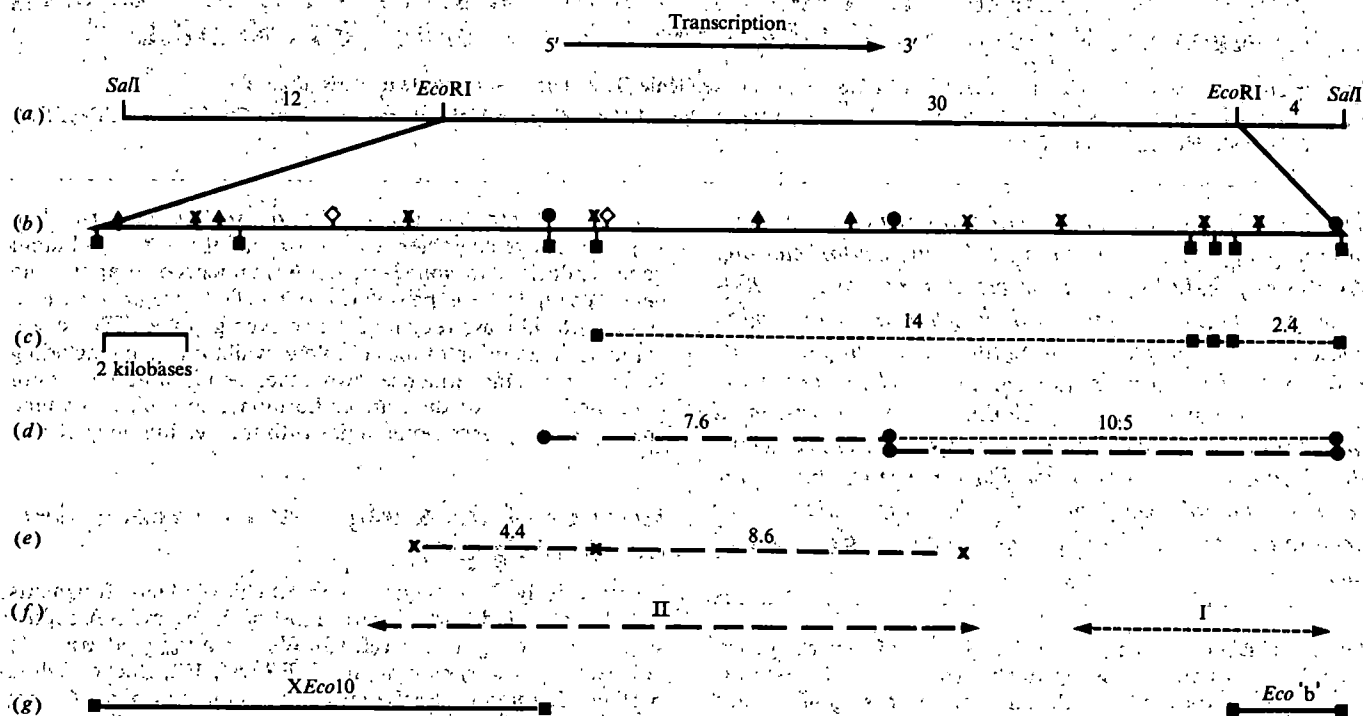
## Isolation of DNA fragments overlapping the ovalbumin gene

We previously<sup>7,8,11</sup> cloned four *EcoRI* chicken DNA fragments ('a', 'b', 'c' and 'd'), which carry most of the ov-mRNA coding sequences (see Fig. 1c and ref. 10). We next attempted to isolate adjacent regions by cloning, in  $\lambda$ WES (ref. 17), chicken DNA partially digested by *EcoRI* (A.G. *et al.*, unpublished). As this approach failed, we undertook to develop a new cloning system capable of propagating large pieces of DNA (F.B. *et al.*, in preparation). Before these experiments were completed, similar cloning vehicles, named 'cosmids', were described by Collins and Hohn<sup>18</sup>. Cosmids are plasmid vehicles which can be packaged *in vitro* into phage  $\lambda$  heads. In this reaction, recombinant molecules are produced in preference to parental molecules and are efficiently converted into infectious particles which can then inject their DNA into bacteria, thus giving rise to bacterial clones harbouring recombinant plasmids. As an efficient screening method was a prerequisite for the isolation of genes from complex genomes by this method, we developed an appropriate *in situ* colony hybridisation technique<sup>19</sup>.

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**Fig. 1** Restriction enzyme map of the cloned pAR1 DNA. pAR1 was constructed as follows: chicken erythrocyte DNA was partially digested with *MboI* and fractionated by sedimentation in a sucrose gradient (5–40% sucrose in 10 mM Tris-HCl pH 8, 1 mM EDTA, 1 M NaCl; sedimentation for 18 h at 25,000 r.p.m. at 4°C in a SW41 Beckman rotor). Fractions were analysed by agarose gel electrophoresis and those containing fragments of 15 to 25 kilobases were pooled. DNA was concentrated by ethanol precipitation and 5 µg were ligated to an equimolar amount of pJC74Km DNA digested by *BamHI* as previously described<sup>7</sup>. (We constructed pJC74Km by *in vitro* recombination of *EcoRI* linearised pJC74 (ref. 20) with a 7-kilobase *EcoRI* fragment of pML2 (ref. 21) containing a kanamycin resistance gene.) *In vitro* packaging<sup>18</sup> yielded 250,000 recombinants. After infection of *E. coli* strain 1,400 (ref. 19) bacteria were spread on kanamycin plates and screened by *in situ* colony hybridisation with an ovalbumin-specific <sup>32</sup>P-labelled probe (*XbaI*-*HindIII* probe, shown in line *d*, and derived from cloned ovalbumin *EcoRI* fragment 'b'). A total of 25,000 colonies was examined and one positive clone was obtained. pAR1 plasmid was re-isolated three times. A restriction map of pAR1 was constructed by analysing restriction enzyme digests of pAR1 DNA: *a*, Linear map of pAR1 showing the 17-kilobase *MboI* chicken DNA fragment integrated in the *BamHI* site of pJC74Km; *b*, Enlargement of the 17-kilobase chicken DNA fragment, showing the restriction sites for *BamHI* (▲), *EcoRI* (■), *KpnI* (●) and *XbaI* (×); *c*, Map of the ovalbumin gene<sup>10,16</sup> showing the introns (identified by letters A–G), the exons (numbered 1–7) and the leader-coding sequence (L). The boundaries of *EcoRI* fragments 'a', 'b', 'c' and 'd' are indicated above the line; *d*, Region corresponding to the ovalbumin-specific probe *XbaI*-*HindIII*. Also shown are the direction of transcription and a scale for lines *b* and *c*.



**Fig. 2** Restriction enzyme map of the cloned pAR2 DNA. Recombinants were constructed as described in Fig. 1, except that chicken DNA was partially digested with *EcoRI*, and that fragments of more than 25 kilobases (25 µg) were pooled and recombined *in vitro* with 15 µg of *EcoRI*-linearised pJC74 in a final volume of 140 µl. 2 µg of recombinant DNA were packaged *in vitro*. After infection of strain 1,400 and spreading on ampicillin plates, 20,000 colonies were screened with the *XbaI*-*HindIII* probe (see Fig. 1). One positive clone was obtained. Its restriction map was derived from a series of digests performed on whole pAR2 DNA or isolated (subcloned) fragments. It was completed by the identification of fragments which hybridise with an *XEco10* probe (see below and text) or the ovalbumin probe *XbaI*-*HindIII* (see Fig. 1). *a*, Map of pAR2 DNA linearised with *SalI*, showing the location of the 30-kilobase insert within the vector sequences. The sizes (in kilobases) of the vector or chicken DNA fragments are indicated above the line. *b*, Map of the *BamHI* (▲), *EcoRI* (■), *HpaII* (◇), *KpnI* (●) and *XbaI* (×) sites in the cloned fragment. *c*, *d* and *e*, maps of the cellular DNA fragments detected in the Southern transfer experiment described in Fig. 3 with either probe I (---) or probe II (—) (see Fig. 3). Digestion by *EcoRI* (*c*), *KpnI* (*d*) or *XbaI* (*e*). The size (in kilobases) is indicated above each fragment. *f*, Probes used for comparison of pAR2 to cellular DNA (Fig. 3): Probe I corresponds to a mixture of the 4.5-kilobase *PstI* and the 3.2-kilobase *EcoRI* fragments in the ovalbumin leader region, isolated from AC4-ov5 (ref. 16). Probe II corresponds to a 14-kilobase long *HhaI* fragment of pAR2 which was subcloned by blunt end ligation into pBR322. *g*, Location of the ovalbumin *EcoRI* fragment 'b' and of the cloned 10.5 kilobase *EcoRI* fragment *XEco10*. This fragment, which was cloned independently in *λ*WES, was selected after hybridisation with the ovalbumin *HhaI* probe<sup>8</sup> and will be described elsewhere (J.L.M. *et al.*, in preparation). The scale (for lines *b*–*g*) is given in line *c* in kilobases.



Two cosmids were used: pJC74 (from J. Collins) which can accept foreign DNA fragments of up to 32 kilobases<sup>20</sup> and pJ74Km, a kanamycin-resistant derivative which we constructed (see Fig. 1 legend) and which can accept DNA fragments of up to 25 kilobases. Chicken erythrocyte DNA, partially digested by *Mbo*I and sized on a sucrose gradient, was ligated to pJC74Km DNA made linear using *Bam*HI. *Bam*HI and *Mbo*I have identical cohesive ends which allow their ligation. In other experiments, chicken erythrocyte DNA, partially digested by *Eco*RI, was sized on sucrose gradients, and recombined with *Eco*RI-linearised pJC74 DNA. After *in vitro* packaging, infection of *recA*<sup>-</sup> strain 1400 (ref. 19), and screening by *in situ* hybridisation with a <sup>32</sup>P-labelled probe corresponding to part of ovalbumin *Eco*RI fragment 'b' (see Fig. 1d), we isolated two clones, from which the recombinant plasmids pAR1 (derived from pJC74Km) and pAR2 (derived from pJC74) were purified.

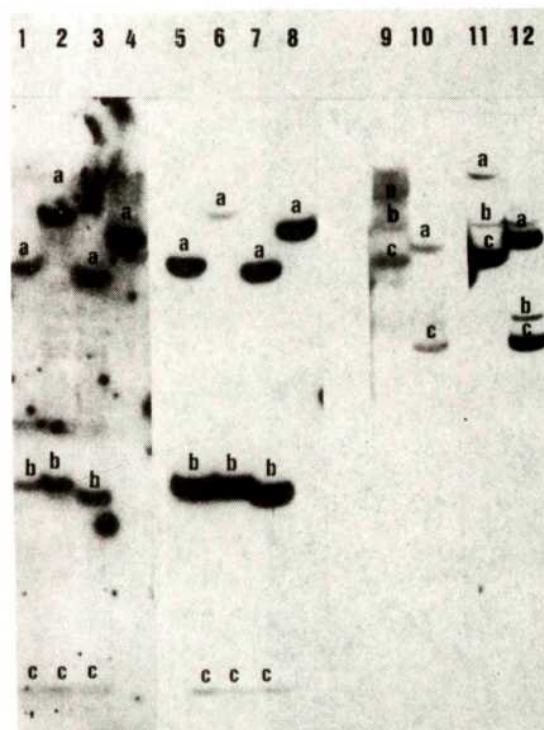
### The cloned sequences cover 46 kilobases in the region of the ovalbumin gene

Several restriction enzymes were used to construct the maps of pAR1 and pAR2 shown in Figs 1 and 2. Digestion of pAR1 DNA by *Eco*RI yielded eight fragments, including a 9.2-kilobase and a 1.3-kilobase fragment, which co-migrated with the cloned *Eco*RI fragments 'a' and 'd' of the ovalbumin gene<sup>7,11</sup> (see Fig. 1b, c). The part of the map containing these two fragments confirmed our previous data on the ovalbumin gene organisation<sup>5,7-11</sup>. There was no fragment of the size of *Eco*RI fragment 'b', but restriction mapping indicated that about half of its sequence was present, as expected as pAR1 was isolated using a probe corresponding to that region. This was further demonstrated by hybridisation of pAR1 DNA with ov-mRNA. Under the electron microscope (not shown), DNA loops corresponding to introns D, E, F and G were observed, in addition to a non-hybridised RNA tail corresponding to exons 1 and 2. From these experiments we conclude that pAR1 contains about 17 kilobases of chicken DNA, starting from the *Mbo*I site located within intron C (refs 8, 10), and ending 12.5 kilobases downstream from the 3' end of the ov-mRNA coding sequence (Fig. 1).

Digestion of pAR2 DNA by *Eco*RI yielded a series of fragments among which the ovalbumin *Eco*RI fragment 'b' (which contains exons 1 to 4) could be identified in a Southern transfer experiment<sup>22</sup> using an ovalbumin double-stranded cDNA probe (*Hha*ov, see refs 5, 23), while fragments 'a', 'c' and 'd' (which contain exons 5, 6 and 7) could not be detected (not shown). Unexpectedly, a 3.5-kilobase *Eco*RI fragment and a 2.4-kilobase *Bam*HI fragment, which were not predicted from the ovalbumin gene map, also reacted with the *Hha*ov probe (see below and Fig. 6). Similar *Eco*RI and *Bam*HI fragments which cross-hybridise with the *Hha*ov probe, but do not belong to the ovalbumin gene, have been detected previously in cellular DNA<sup>9</sup>. This suggests that pAR2 contains some of these ovalbumin-related sequences. The restriction map in Fig. 2 (a and b) indicates that pAR2 contains about 30 kilobases of chicken DNA located on the 5' side (with respect to transcription) of the ovalbumin gene (see Figs 1c, 2g and ref. 16). As will be shown below, there are sequence homologies between the ovalbumin gene region which is on the 3' side of fragment *Eco* 'b' and two other genes, X and Y, which are present in pAR2.

In view of the large size of the DNA fragment which was integrated into pAR2 and because it is the first example of the use of cosmids for cloning eukaryotic DNA, it was important to demonstrate that this cloned fragment exists as such in genomic DNA. We have compared the hybridisation patterns of restriction enzyme digests of pAR2 and chicken oviduct DNA in Southern transfer experiments. Two probes were used for hybridisation (Fig. 2f, g). Probe I covers 6 kilobases in the ovalbumin *Eco*RI fragment 'b' region. Probe II covers 14 kilobases in the middle of the cloned DNA. As shown in Fig. 3, the hybridisation patterns of pAR2 and oviduct DNAs with probe I

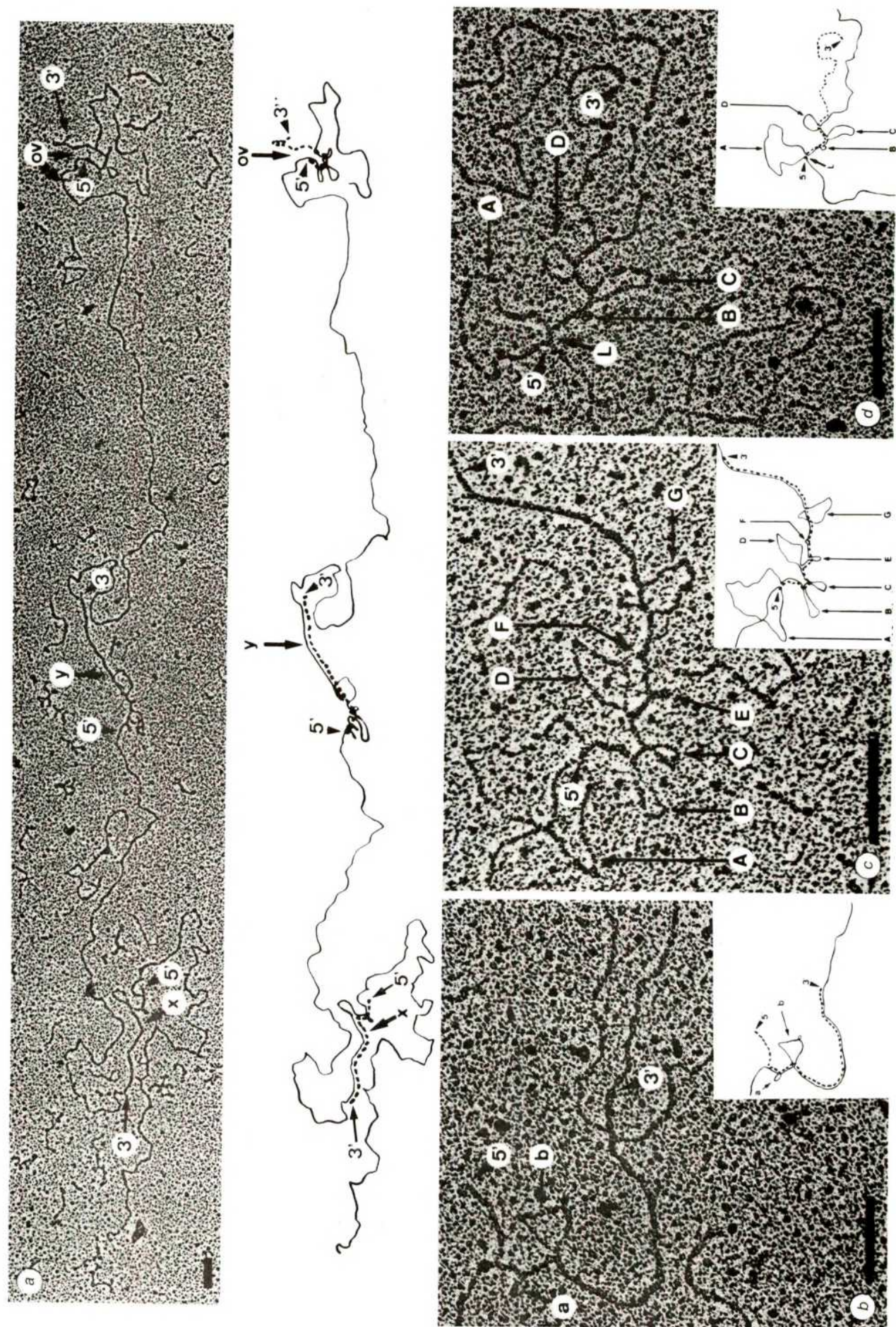
are identical in digests made with *Eco*RI (lanes 2 and 6) or *Kpn*I (lanes 4 and 8), or in double digests made with *Eco*RI and either *Bam*HI (lanes 1 and 5) or *Kpn*I (lanes 3 and 7). There are two *Xba*I fragments of similar size in pAR2 and oviduct DNA which hybridise to probe II (lanes 10 and 12). This probe also reacts with three fragments in *Kpn*I digests of both DNAs (lanes 9 and 11). Two of these fragments (bands b and c) have similar sizes in pAR2 and oviduct DNAs. In pAR2, a third fragment of about 26 kilobases which contains 16 kilobases of vector sequences is observed (band a, lane 11), while the corresponding fragment in cellular DNA has a size of about 18 kilobases and contains an



**Fig. 3** Restriction enzyme mapping of cloned pAR2 DNA and of the corresponding chromosomal DNA region. pAR2 DNA or oviduct DNA were digested with various restriction nucleases and electrophoresed in 0.7% agarose gels (10 µg of cellular DNA or 10 ng of pAR2 DNA per slot). Transfer on to nitrocellulose filters, hybridisation conditions and autoradiography were as previously described<sup>22</sup>. Digestions which were to be analysed with the same probe were all electrophoresed on the same gel, but cellular DNA and pAR2 DNA slots were transferred on to separate filters. Hybridisation of oviduct DNA (lanes 1-4) or pAR2 DNA (lanes 5-8) with nick-translated probe I (see Fig. 2). Fragment sizes are given in kilobases. Lanes 1 and 5: digestion with *Bam*HI and *Eco*RI, a = 7.9, b = 2.4, c = 0.5. Lanes 2 and 6: digestion with *Eco*RI, a = 14, b = 2.4, c = 0.5. (The band above fragment 'b' which appears in lanes 1 and 2 is an artefact.) Lanes 3 and 7: digestion with *Eco*RI and *Kpn*I, a = 7.2, b = 2.2, c = 0.5. Lanes 4 and 8: digestion with *Kpn*I, a = 10.5. Hybridisation of oviduct DNA (lanes 9 and 10) or pAR2 DNA (lanes 11 and 12) with nick-translated probe II (see Fig. 2). Lanes 9 and 11: digest with *Kpn*I, a (lane 9) = 18, a (lane 11) = 26, b = 10.5, c = 7.6. Lanes 10 and 12: digest with *Xba*I, a = 8.6, b = 5.1, c = 4.4. Band b (lane 12) corresponds to the *Xba*I fragment adjacent to fragment 'c' (at its left, see Fig. 2b, e). As fragment 'b' hybridises only to a very small portion of probe II, it was not detected in the cellular DNA.

additional 8-kilobase segment not present in pAR2 (band a, lane 9). As shown in Fig. 2c, d and e, the fragments hybridising to probes I and II and which are common to oviduct and pAR2 DNA, cover a 24-kilobase segment. In addition, the 10.5-kilobase region of the cloned DNA which is distal to the ovalbumin sequence, and which overlaps this 24-kilobase segment, has been found to be identical to an independently cloned chicken DNA fragment XEco10 (see Fig. 2g) which has been studied separately (J.L.M. *et al.*, in preparation). From all these results we conclude that the entire 30-kilobase region cloned in pAR2 corresponds to a single continuous segment of cellular DNA, which has not suffered any major rearrangement during cloning.







## pAR2 contains the ovalbumin leader-coding sequence

As pAR2 contains a very large segment of DNA on the 5' side of the ovalbumin gene, we expected that it should have the ov-mRNA leader-coding sequence<sup>10</sup>. We have analysed by electron microscopy the hybrid molecules formed between ovalbumin mRNA and heat-denatured pAR2 DNA, previously linearised by *SalI* (Fig. 4d). ov-mRNA hybridises over  $620 \pm 67$  nucleotides in five regions separated by four single-stranded DNA loops. Three of these loops have sizes and locations corresponding to introns B, C and D of the ovalbumin gene which allows the orientation of the molecule with respect to transcription<sup>8,9</sup>. The fourth DNA loop which is at the 5' end of ov-mRNA is  $1,606 \pm 150$  nucleotides long (Fig. 4d). This loop corresponds to intron A, which was expected to separate exon 1 from the sequence coding for the mRNA leader<sup>10</sup>. Indeed both the length of loop A and the location of several restriction sites in the corresponding region of pAR2 agree with data obtained on two other independently isolated clones where the presence of the leader-coding sequence at the 5' end of the loop was unequivocally demonstrated by DNA sequencing<sup>16</sup>. The free mRNA tail, which is about 1,200 nucleotides long, corresponds to exons 5, 6 and 7 which are not present in pAR2.

## pAR2 contains two other genes expressed in oviduct of laying hen

In the 46-kilobase DNA region cloned in pAR1 and pAR2, the ovalbumin gene occupies only about 7.7 kilobases<sup>16</sup>. This could leave ample space for other genes. As we were interested to know whether genes which share a similar hormonal regulation of expression in chicken oviduct could be clustered, we have hybridised total poly(A) RNA from laying hen oviducts with pAR1 or pAR2 DNAs. No RNA other than ov-mRNA was found to hybridise to pAR1 DNA. In contrast, electron microscopy of the hybrid molecule revealed that, besides ov-mRNA, two other RNA molecules hybridised to the same strand of pAR2 DNA (Fig. 4a), indicating the presence of three genes transcribed in the same direction (Fig. 4a). The polarity with respect to transcription is given by the ov-mRNA-DNA hybrid region which is easily identified by the characteristic array of DNA loops corresponding to introns B, C and D (Fig. 4a, d, see below for the absence of the loop corresponding to intron A in Fig. 4a). We termed these two other genes, X and Y, giving the gene order 5'-X-Y-ovalbumin-3'.

Y RNA is  $2,020 \pm 175$  nucleotides long, as measured from the length of the DNA-RNA hybrid regions located in the middle of the cloned segment. The DNA loops indicate the presence of seven introns (A to G) in this gene, separating eight exons (1 to 8) (Figs 4c and 5a, b). Note that with Y RNA, as with ov-mRNA, the DNA loop closest to the 5' end of the RNA is not seen on all hybrid molecules (and is in fact absent for both genes in Fig. 4a). In the case of ov-mRNA this is due to the short size (47 nucleotides) of the leader sequence which does not form a very stable hybrid in the conditions used to form preferentially

DNA-RNA hybrids<sup>16</sup>. A similar situation is probably encountered with Y RNA, as its exon 1 is not actually resolved by electron microscopy and its existence is inferred from the presence of loop A (Fig. 4c). The total length of the Y gene (exons + introns) is about 6.5 kilobases, and its 3' end lies about 11.5 kilobases upstream from the ov-mRNA leader-coding sequence.

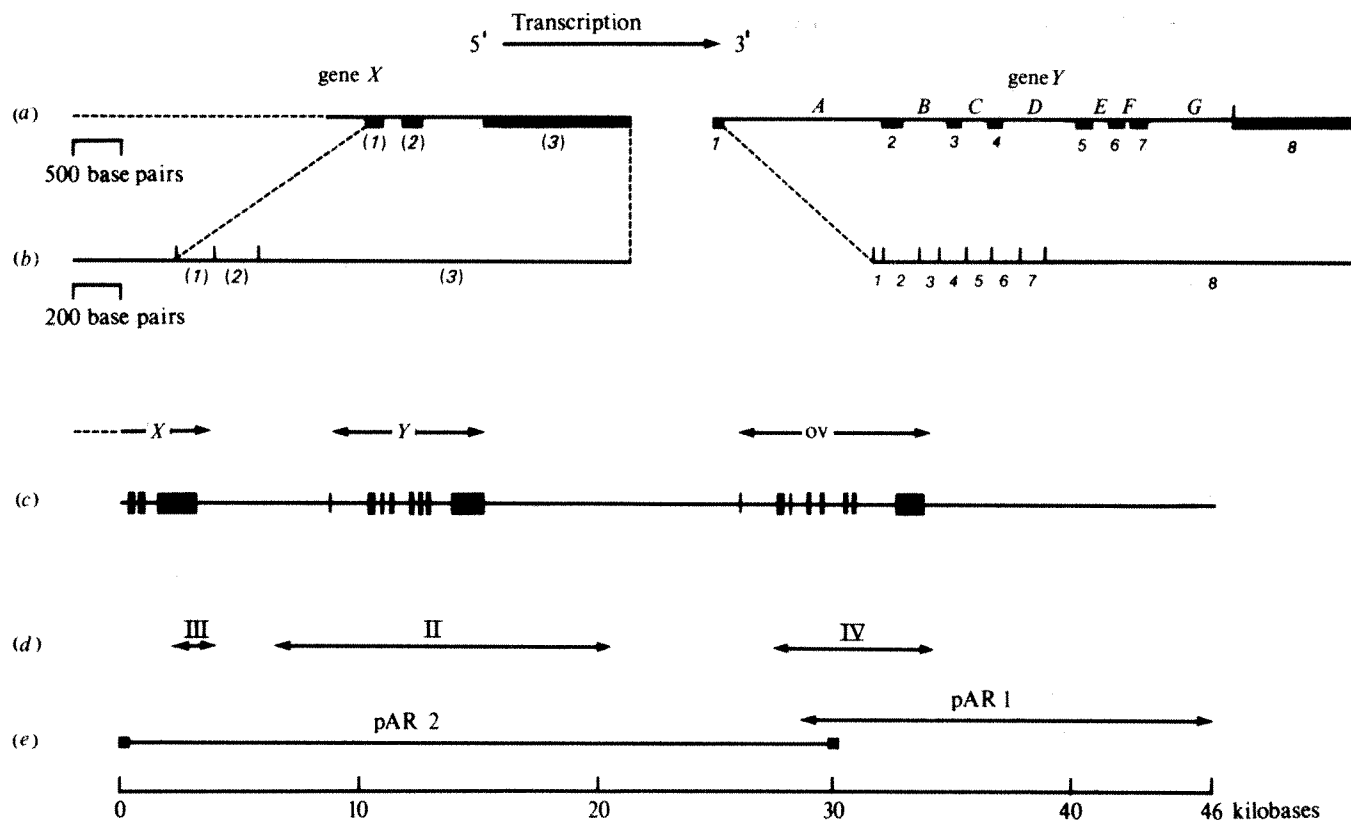
X RNA is about 2,400 nucleotides long. It hybridises to pAR2 DNA over  $1,945 \pm 200$  nucleotides in three exons separated by two DNA loops (introns) (Figs 4b and 5a, b). The presence of a free RNA tail of about 400 nucleotides indicates that the region coding for the 5' end of this RNA is not present in pAR2 DNA. There are about 5.5 kilobases between the 3' end of the X gene and the 5' end of the Y gene. Using these data, genes X and Y were positioned on the restriction enzyme map as shown in Figs 5c and 6B(I).

## X, Y and ovalbumin genes share sequence homologies

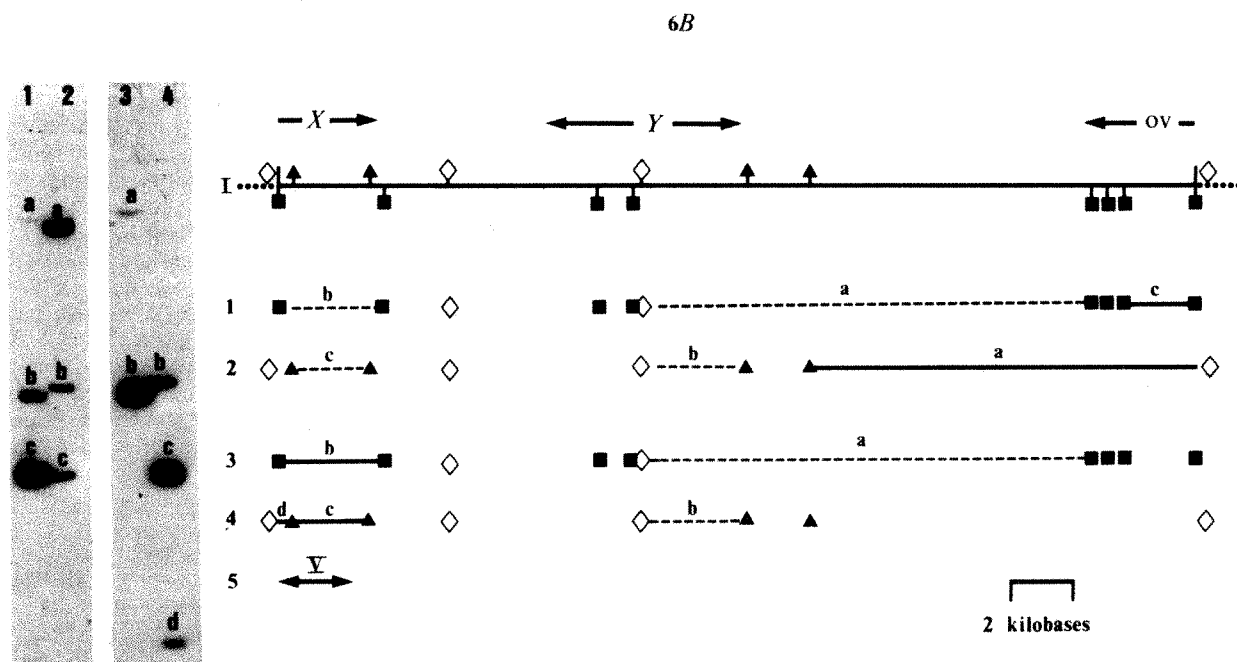
In the original mapping of pAR2 DNA (see above) we found that, in addition to the ovalbumin gene sequences, some other parts of the DNA hybridised with the ovalbumin-specific *Hha*ov probe<sup>5,23</sup>. The finding of genes X and Y prompted us to investigate whether the cross-hybridising regions could correspond to these genes.

pAR2 DNA was digested by *Hpa*II and either *Eco*RI or *Bam*HI. *Hpa*II cuts the cosmid DNA into very small fragments, facilitating further analysis of the cloned chicken DNA, which is cut only two times. Digestion of the DNA from pAR2 with *Hpa*II and *Eco*RI yields one band (band c, lane 1, Fig. 6A) which hybridises strongly with the *Hha*ov probe. Two other bands of weaker intensity (bands a and b, lane 1) were also revealed. From the restriction enzyme mapping of pAR2 we can unambiguously identify these bands and show their location in Fig. 6B, line 1. Similarly, in a *Hpa*II and *Bam*HI double digest, hybridisation with the *Hha*ov probe yielded one intense band (band a, lane 2, Fig. 6A), which includes the ovalbumin coding region and two other bands (bands b and c, lane 2), which are located in the regions of the DNA coding for genes Y and X, respectively (see Fig. 6B, line 2). These homologies were further analysed using a probe from the region of gene X (probe V, Fig. 6B, line 5). On digestion with *Hpa*II and *Eco*RI, two bands of hybridisation were obtained (lane 3, Fig. 6A): band a which includes part of gene Y, and band b which contains the region from which the probe was prepared (see Fig. 6B, line 3). Using the same probe with DNA digested by *Hpa*II and *Bam*HI, three bands of hybridisation were found (Fig. 6A, lane 4). Two of these bands (c and d) originate from gene X, whereas band b corresponds to a part of gene Y (see Fig. 6B, line 4). These experiments show that there are homologies between regions of genes X and Y, and that both genes contain sequences which are homologous to some ovalbumin exonic sequences. Even so, these three genes and their RNA transcripts are clearly different, as indicated by the electron microscopic results and by

**Fig. 4** Electron micrographs of RNA-DNA hybrid molecules between laying hen oviduct RNA purified on oligo(dT)-cellulose and pAR2 DNA. RNA-DNA hybrids were formed by incubating heat-denatured linear pAR2 DNA ( $1.5 \mu\text{g ml}^{-1}$ ) with oligo(dT)-cellulose-purified RNA ( $100 \mu\text{g ml}^{-1}$ ) at  $54.3^\circ\text{C}$  and prepared for electron microscopy<sup>7</sup>. pAR2 DNA was linearised by digestion with *SalI*. In the line drawings, the RNAs are represented as a dashed line and the DNAs as a solid line. Scale bars represent  $0.1 \mu\text{m}$ . **a**, Electron micrograph of a complete single-stranded pAR2 DNA molecule hybridised with three RNA molecules. Arrows X, Y and ov point to the respective genes. Arrow heads 5' and 3' indicate the 5' and the 3' extremities of the RNAs. The total length of pAR2 DNA is  $45,940 \pm 6,600$  base pairs (from the measurements of 20 molecules). The X RNA hybridises to a DNA sequence which lies  $10,460 \pm 1,150$  base pairs from the nearest pAR2 DNA end. There are  $5,560 \pm 600$  base pairs between the 3' end of the X gene and the 5' end of the Y gene and  $11,580 \pm 1,350$  base pairs between the 3' end of the Y gene and the 5' end of the ovalbumin gene. There are  $5,330 \pm 540$  base pairs between the DNA sequence which hybridises to ov-mRNA and the end of the molecule. Loops A of the ovalbumin gene (see Fig. 4b) and of the Y gene (see Fig. 4c) are not visible in this picture, presumably because the hybrids responsible for their presence are not very stable (see text). **b**, Electron micrograph of an X RNA-DNA hybrid molecule. The hybrid region is  $1,945 \pm 243$  base pairs long (from the measurements of 20 molecules). The free RNA tail is about 450 nucleotides long. The two single-stranded DNA loops 'a' and 'b' are  $253 \pm 47$  and  $812 \pm 120$  nucleotides long, respectively. The hybrid segments are (from 5' to 3')  $175 \pm 39$ ,  $181 \pm 35$  and  $1,613 \pm 219$  base pairs, respectively. Other symbols as under (a). **c**, Electron micrograph of a Y RNA-DNA hybrid (from the measurements of 20 molecules). The hybrid region is  $2,020 \pm 175$  base pairs. The length of the single-stranded DNA loops A to G are  $1,729 \pm 180$ ;  $492 \pm 130$ ;  $303 \pm 88$ ;  $812 \pm 135$ ;  $206 \pm 86$ ;  $55 \pm 20$  and  $890 \pm 134$  nucleotides long, respectively. The hybrid segments (from 5' to 3') are  $218 \pm 71$ ;  $93 \pm 30$ ;  $165 \pm 40$ ;  $153 \pm 33$ ;  $167 \pm 33$ ;  $183 \pm 41$  and  $1,320 \pm 148$  base pairs, respectively. Other symbols as under (a). **d**, Electron micrograph of an ovalbumin mRNA-DNA hybrid (from the measurements of 22 molecules). The hybrid region is  $620 \pm 67$  base pairs long. The free RNA tail is about 1,100 nucleotides long. Loops A to D are  $1,606 \pm 150$ ;  $204 \pm 47$ ;  $578 \pm 95$  and  $386 \pm 85$  nucleotides long. The hybrid segments are (from 5' to 3')  $213 \pm 45$ ,  $76 \pm 23$ ,  $171 \pm 33$  and  $162 \pm 46$  base pairs long. The L arrow points to the leader-coding segment which is revealed by the presence of loop A. Other symbols as under (a).



**Fig. 5** Map of the ovalbumin gene region. *a, b*, Maps of genes *X* and *Y* indicating the respective locations of introns (designated by letters), exons (heavy lines and numbers) which are also shown on the maps of the corresponding RNAs (line *b*) where they are designated by numbers (for gene *X* the numbers are in brackets as definitive numbering of exons requires knowledge of the complete structure of the gene). *c*, Map of the 46-kilobase DNA region contained in pAR1 and pAR2, showing the location of the exons (heavy lines) of *X*, *Y* and ovalbumin (*ov*) genes. *d*, Probes used for the experiment in Fig. 7. Probe II is described in Fig. 2 legend. Probe III is a 1.8-kilobase *Hind*III fragment isolated from *XEco*10 (see Fig. 2 legend). Probe IV corresponds to the ovalbumin *Eco*RI fragment 'b' and 'c' and to a part of *Eco*RI fragment 'a' (see Fig. 1c). *e*, Limits of the fragments integrated in pAR1 and pAR2 and scale (in kilobases) for lines *c*, *d* and *e*.



**Fig. 6** Sequence homologies between *X*, *Y* and ovalbumin genes. *A*, Digests of pAR2 DNA with *Eco*RI and *Hpa*II (lanes 1 and 3) or *Bam*HI and *Hpa*II (lanes 2 and 4) were electrophoresed on a 0.7% agarose gel, transferred on to nitrocellulose filters and hybridised to probes corresponding to the ovalbumin gene (*Hhaov*: lanes 1 and 2) or to gene *X* (probe V, lanes 3 and 4). The latter probe, shown in line 5 of Fig. 6B is a 2.3-kilobase *Hind*III fragment subcloned from *XEco*10 (see Fig. 2 legend). The size of the fragments are (in kilobases): lanes 1 and 3, *a* = 14, *b* = 3.4, *c* = 2.4; lanes 2 and 4, *a* = 12.5, *b* = 3.6, *c* = 2.4 and *d* = 0.9. *B*, The location of the fragments hybridising in lanes 1 to 4 of Fig. 6A are indicated in lines 1 to 4, respectively. For a given probe the continuous line shows the fragment(s) which contain(s) part or all of the sequences of the probe, whereas dashed lines indicate additional fragments which cross-hybridise with the probe. The first line (I) gives the location of *Bam*HI (▲), *Eco*RI (■) or *Hpa*II (◇) restriction sites and indicate the regions corresponding to genes *X*, *Y* and ovalbumin. The dotted line corresponds to vector sequences. Line 5: location of probe V and scale.

the lack of hybridisation of the *Y* gene with the *Xba*I–*Hind*III probe (Fig. 1d) prepared from the ovalbumin *Eco*RI fragment 'b' (not shown).

### Expression of *X* and *Y* is under hormonal control

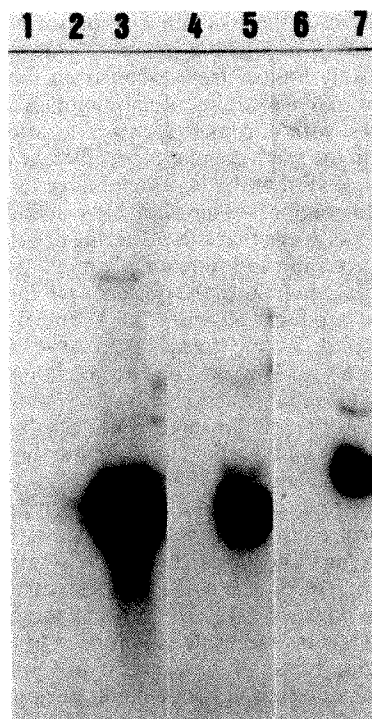
As transcription of the ovalbumin gene is under oestrogen control, we wondered whether the expression of *X* and *Y* is regulated in a similar way. Oviduct RNA from laying hen or oestrogen-withdrawn chicken was fractionated on methyl mercuric hydroxide agarose gels, transferred and immobilised on to diazobenzoyloxymethyl paper<sup>24</sup>. The filters were hybridised with probes specific for each of the three genes.

As shown in Fig. 7, probe IV (see Fig. 5d), specific for the ovalbumin gene, hybridised with an RNA molecule about 1,900 nucleotides long, which is present in laying hen oviduct (lane 3) but cannot be detected in 'hormone-withdrawn' oviduct (lane 1), as expected for ovalbumin mRNA. Probe III (see Fig. 5d) is specific for gene *X* as it does not contain the regions which cross-hybridise with either *Y* or ovalbumin genes (J.L.M. *et al.*, in preparation). An RNA species present in laying hen (Fig. 7, lane 7), but not in 'hormone-withdrawn' oviduct (lane 6) hybridises to this probe. As expected from the above electron microscopic results, the size of this RNA is about 2,300 nucleotides. Gene *Y* is not expressed in the 'hormone-withdrawn' oviduct (Fig. 7, lane 4) as no RNA species hybridised with probe II which contains gene *Y* (see Fig. 5d). In laying hen oviduct (lane 5) we found an RNA species, about 2,000 nucleotides long, which hybridised with probe II. But we cannot state with certainty that it is RNA *Y*, because probe II also cross-reacts with ov-mRNA, which is of similar size. However, the electron microscopic results indicate unambiguously that RNA *Y* is present in the oviduct of laying hens. That the transcription of *X* and *Y* genes is oestrogen-dependent is indicated by the presence of *X* and *Y* RNAs in oviducts following administration of oestradiol to 'withdrawn' chicken (not shown). However, the results shown in Fig. 7 and the frequency of RNA-DNA hybrids observed for *X* and *Y* genes by electron microscopy suggest that the concentration of *X* and *Y* RNAs is much lower than that of ov-mRNA.

### Organisation of the chicken genome in the vicinity of the ovalbumin gene

Cloning of eukaryotic DNA fragments using cosmid vectors has not been described previously. Our experiments show that this system, in conjunction with an appropriate *in situ* hybridisation method<sup>19</sup>, can be used successfully. Available phage vectors cannot propagate more than about 20 kilobases<sup>17,25</sup>. Using pJC74 cosmid, we have cloned a 30-kilobase DNA segment of the chicken genome. Shorter cosmid vectors should permit the cloning of DNA fragments of up to 40–45 kilobases. As such large DNA fragments have a greater chance of containing repeated sequences and might be unstable during cloning, we used a *recA*<sup>–</sup> bacterial host (some phage vectors, including  $\lambda$ Charon 4A (ref. 25), would not grow on such hosts). The efficiency of the cosmid vector system is such<sup>18,20</sup> that it is possible to construct a genomic library from a few microgrammes of eukaryotic DNA. Enrichment for a particular gene sequence before cloning seems to be unnecessary and even undesirable, as it might lead one to discard fragments of unexpected interest, such as genes of related sequences.

In one clone we found two other genes (*X* and *Y*) closely linked to the ovalbumin gene. As the existence of genes *X* and *Y* was revealed by electron microscopy of hybrid molecules between total oviduct poly(A)<sup>+</sup> RNA and the cloned DNA, we would not have detected genes coding for minor species of oviduct RNAs or RNAs expressed in other tissues. Assuming that no other genes are present in the cloned DNA, the length of the intergene regions (5–10 kilobases) and of the genes themselves (6–8 kilobases), is such that there would be one gene every 10 to 15 kilobases of DNA.



**Fig. 7** Detection of *X* and *Y* RNAs in oviducts of laying hen and 'withdrawn' chicken. Female chicks aged 5 days were injected daily with 1 mg of oestradiol benzoate for a period of 10 days (primary stimulation). Hormone treatment was then stopped and 4 weeks later the magnum portion of the oviduct was removed ('withdrawn' oviduct). Total RNA from laying hen or hormone-withdrawn oviduct was prepared according to a method developed by Dr F. Rougeon (personal communication). RNA samples (20  $\mu$ g per slot) were separated by electrophoresis on 1% agarose gels containing 10 mM methyl mercury hydroxide. The RNA was then transferred to diazobenzoyloxymethyl paper which was hybridised to <sup>32</sup>P-labelled probes and washed<sup>24</sup>. Autoradiography was with a Kodirex film (Eastman Kodak) for 4 days. Lanes 1–3: hybridisation to  $2.5 \times 10^6$  d.p.m. of <sup>32</sup>P-labelled probe IV (see Fig. 5d); lanes 4 and 5: hybridisation to  $2.5 \times 10^6$  d.p.m. of <sup>32</sup>P-labelled probe II (see Figs 2f and 5d); lanes 6 and 7: hybridisation to  $4 \times 10^6$  d.p.m. of <sup>32</sup>P-labelled probe III (see Fig. 5d); lanes 1, 2, 4 and 6: 20  $\mu$ g of total oviduct RNA from 'withdrawn' chicken; in lane 2, 0.1  $\mu$ g of laying hen oviduct RNA was added; lanes 3, 5 and 7: 20  $\mu$ g of total RNA from laying hen oviducts.

As *X*, *Y* and ov-mRNAs are produced in differentiated cells, could reorganisation of the genome in this region occur during differentiation, as in the case of immunoglobulin genes? (See refs 26 and 27 and refs therein.) Our comparison of the restriction map of the erythrocyte DNA cloned in pAR2 and of either oviduct DNA (Fig. 3) or erythrocyte DNA (results not shown) indicates that there is no major rearrangement.

There are striking resemblances (Figs 4 and 5c) in the organisation of these neighbouring genes, which are transcribed from the same strand and are under oestrogen control. More than half of the RNA-coding sequences are found uninterrupted at the 3' end of all three genes. For both the ovalbumin and *Y* genes there are seven introns and the eight regions coding for the mature RNAs are very similar in size. In addition, the first intron (intron A) is about 1,600 nucleotides long in the two genes and separates a very short RNA-coding sequence from the neighbouring exon. It will be interesting to find whether exon 1 of gene *Y* is equivalent to the mRNA leader-coding sequence of the ovalbumin gene<sup>16</sup>. The above similarities and the fact that genes *X*, *Y* and ovalbumin share partial sequence homologies strongly suggest that they could have arisen by at least partial duplications from a common precursor. In our previous work (refs 7, 9; see also Fig. 1 in ref. 5) we detected a limited number of cellular DNA fragments in the chicken genome, which cross-hybridised to the *Hha*ov ovalbumin cDNA probe, but did not belong to the ovalbumin gene. Genes *X* and *Y* account for the existence of most, if not all, of these fragments (our unpublished results). The presence in the chicken genome of other genes with the same degree of homology with the ovalbumin gene is therefore unlikely.



The organisation of the ovalbumin region is reminiscent of that found for the  $\beta$ -like globin genes<sup>28,29</sup>. Genes for  $\beta$ - and  $\delta$ -globin are closely linked, transcribed from the same DNA strand, and show extensive sequence homologies in the mRNA coding sequences, with introns in identical locations. They are both expressed in the same cellular type, albeit at very different rates. It is also known that the two  $\gamma$ -globin genes are found in the same genomic region<sup>28</sup>. The available evidence for several gene families<sup>30,31</sup> suggests that the clustering of genes which are related both structurally and functionally might not be uncommon in eukaryotic genomes. In the present case, however, we do not know if the products of genes *X*, *Y* and ovalbumin are functionally related, as the former two have not yet been identified (genes *X* and *Y* do not code for conalbumin, lysozyme or ovomucoid; our unpublished results).

Could the clustering of the three genes play a part in their expression? They could participate together in a chromatin domain (see ref. 32 and refs therein) or be transcribed and controlled by oestrogens as part of one unit. In prokaryotes, it has been demonstrated that genes of related function can be clustered in operons and transcribed by a single RNA-polymerase molecule. There is no evidence at present that this occurs in eukaryotes. In our case, transcription of all three genes by the same RNA-polymerase molecule would have to be reconciled with the following facts: (1) the largest precursor RNA for ovalbumin so far detected is about 8,000 nucleotides<sup>33,34</sup>; (2) there are large differences in the levels of the three RNAs (ov-mRNA being the most abundant); and (3) the clustering of the three genes may have no other significance than reflecting the evolutionary process which gave rise to them.

Biohazards associated with the experiments described in this publication were examined previously by the French National Control Committee. The experiments were carried out in L3-B1 conditions in the nomenclature adopted by the French Committee (*Le Progrès Scientifique* No. 191, Nov/Dec 1977). We thank Dr J. Collins for providing pJC74, Dr B. Hohn for providing strains, information and advice for *in vitro* packaging, Dr P. Courvalin for pML2 DNA, and Dr F. Rougeon for T<sub>4</sub> DNA ligase and useful advice. The technical help of Mrs O. Lebail, A. Hémard, Mrs C. Kutschis, E. Sittler, M. C. Chanal, C. Wasylyk, Mr Garnier and B. Boulay is acknowledged. This work was supported by grants to P.K. from the CNRS (ERA 201,

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## Histone genes are clustered with a 15-kilobase repeat in the chicken genome

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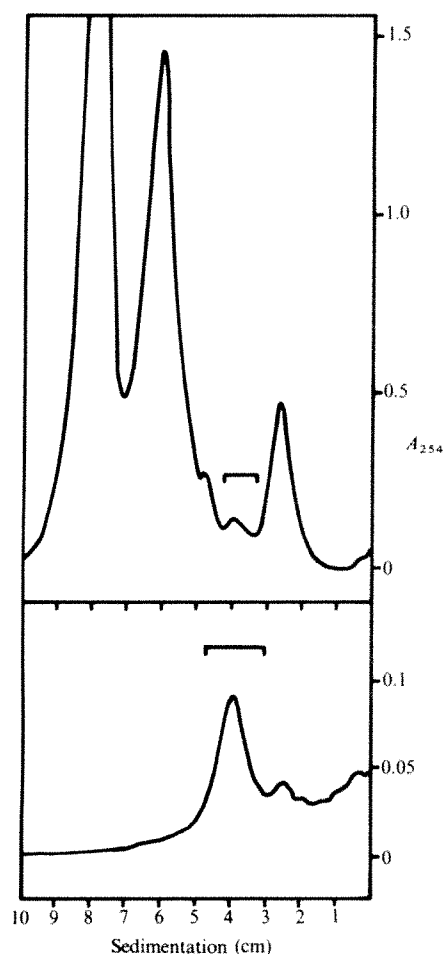
*Histone mRNA isolated from 5-day-old chick embryos has been used as a template for complementary DNA (cDNA) synthesis. The resultant cDNA, after removal of sequences complementary to rRNA, was used to detect histone genes in adult chicken genomic DNA. Hybridisation data indicate that the histone genes are repeated about 10-fold in the chicken genome. Restriction endonuclease analysis reveals some sequence heterogeneity in these genes. However, the results show that chicken histone genes are clustered with a basic repeat unit of 15 kilobases.*

THE organisation of histone genes in sea urchin species has been elegantly demonstrated by mapping restriction endonuclease fragments<sup>1-3</sup> and by electron microscopy<sup>4</sup>. A prerequisite for these studies was the availability of a probe of sufficient radio-

chemical purity to allow unequivocal detection of histone gene sequences. In the sea urchin system, it was possible to take advantage of the fact that early cleavage embryos synthesise predominantly histone mRNA. Pulse-labelled RNA isolated from these cells, although contaminated with other RNA, was perfectly usable because almost all the radioactivity was contained in histone mRNA.

Histone gene sequences from the sea urchin have also been useful in studying *Drosophila* histone genes. Cross reactivity between sea urchin and *Drosophila* histone coding sequences has led to the isolation and subsequent analysis of cloned *Drosophila* histone genes<sup>5</sup>. Other systems have not been so amenable. Despite intensive efforts, isolation of histone mRNA of comparable radiochemical purity from pulse-labelled *Drosophila* tissue culture cells<sup>6</sup> or HeLa cells<sup>7</sup> has been very difficult. Purification of human<sup>8,9</sup> or *Xenopus*<sup>10</sup> histone mRNA sequences by physicochemical methods has similarly proved

elusive. By comparison, 5-day chick embryos seem to be an excellent source of histone mRNA. When cDNA is prepared from this RNA template and sequences complementary to rRNA are removed, the resulting probe can be used to detect histone genes in the genome.



**Fig. 1** Isolation of chick histone mRNA. Five-day-old chick embryos were snap-frozen in liquid  $N_2$ , then homogenised in 7 M guanidinium-Cl in 20 mM Tris-Cl, pH 7.5, 1 mM EDTA and 1% (w/v) Sarkosyl in a Dounce homogeniser in a final volume of 30 ml. Total RNA from this homogenate was recovered as material centrifuged through 5.7 M CsCl as described elsewhere<sup>11</sup>. The clear RNA pellets were gently homogenised in 10 mM Tris-Cl, pH 7.5, 1 mM EDTA, 5% Sarkosyl and 5% phenol, then made 0.1 M in NaCl and phenol extracted with an equal volume of phenol:chloroform (1:1 v/v). RNA from the aqueous phase was collected after precipitation with ethanol, resuspended in 10 mM Tris-Cl, pH 7.4, 1 mM EDTA and 0.5% SDS, heated at 65 °C for 5 min, chilled and centrifuged on 10–40% sucrose gradients at 220,000g for 16 h at 4 °C. The 7–11S RNA was collected and refractionated on a second gradient as shown. Thirty embryos yielded 10–30  $\mu$ g of 7–11S RNA.

### Characterisation of RNA containing histone mRNA

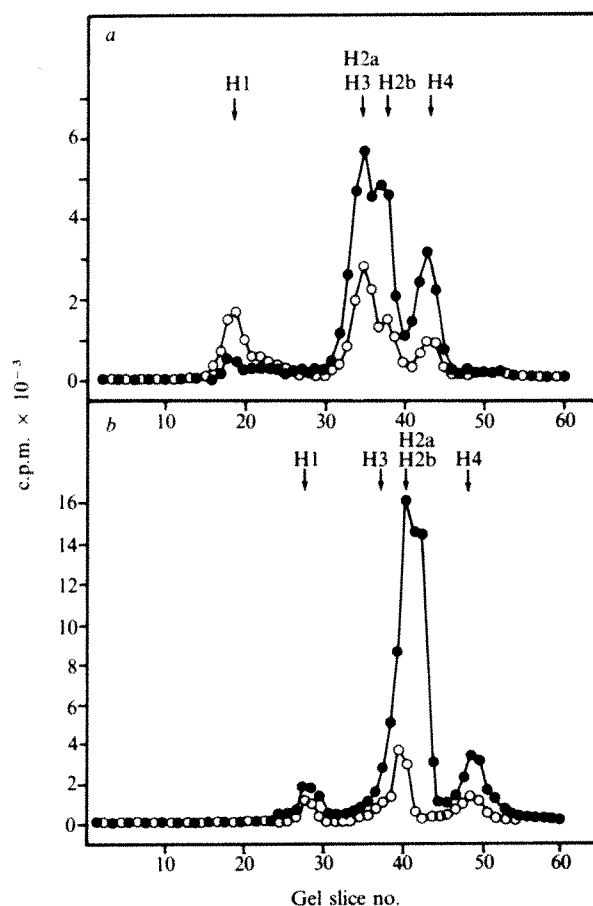
Total RNA was prepared from 5-day-old chick embryos<sup>11</sup>, and a discrete 7–11S RNA species was resolved from other major RNAs by sucrose gradient centrifugation (Fig. 1). This RNA fraction was shown to contain histone mRNA by *in vitro* translation. When the wheat embryo cell-free system<sup>12</sup> was programmed with 7–11S RNA, the five major histones were detected as products. Before Poly(U)–Sepharose chromatography of the RNA, additional products were also detected (data not shown). However, up to 90% of the RNA isolated from the second cycle of sucrose gradient fractionation (Fig. 1) passed

through Poly(U)–Sepharose as unbound material. This RNA programmed the synthesis of histones alone as judged by analysis of protein products on two separate acrylamide-gel electrophoresis systems (Fig. 2). Although it is possible that non-histone translation products (if present) would not enter the low pH gels<sup>14</sup> (Fig. 2b), this would not be the case for SDS-urea gels run near neutral pH (ref. 13) (Fig. 2a). For both systems, the products of *in vitro* translation correspond precisely with marker <sup>14</sup>C-histones electrophoresed in the same gel. These results suggest that the poly(A) minus RNA preparation is free of detectable contaminating mRNA species.

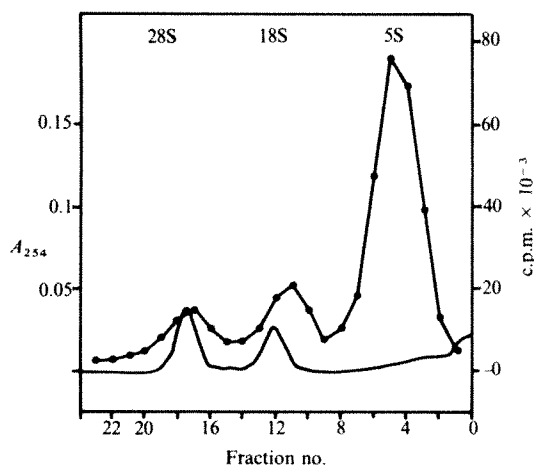
### Characterisation of histone cDNA

Although the 7–11S RNA preparation programmed the synthesis *in vitro* of histones alone, the presence of other kinds of non-polyadenylated RNA without mRNA activity could not be excluded.

It seemed reasonable to assume that rRNA breakdown products would be the most abundant contaminants of the histone mRNAs. Rather than attempt further purification of the RNA sequences themselves, we synthesised randomly primed cDNA<sup>15</sup> and then removed cDNA complementary to rRNA from the mixture. The <sup>32</sup>P-cDNA preparation was hybridised to a vast excess of 28S and 18S rRNA and the mixture centrifuged



**Fig. 2** Analysis on polyacrylamide gels of the *in vitro* translation products of 7–11S RNA unbound to poly(U)–Sepharose. 0.5  $\mu$ g of the unbound 7–11S RNA was translated in the wheat-germ cell-free system of Roberts and Paterson<sup>12</sup> at optimal salt conditions of 80 mM  $K^+$  and 3 mM  $Mg^{2+}$ . <sup>3</sup>H-leucine-labelled translation products were electrophoresed on: a, the SDS-urea gels of Swank and Munkres<sup>13</sup>, and b, the low-pH urea gels of Panyim and Chalkley<sup>14</sup>. The gels were then sliced and the radioactivity measured. ●, <sup>3</sup>H-labelled product of *in vitro* translation; ○, <sup>14</sup>C-labelled chicken histone standards.



**Fig. 3** Preparation of  $^{32}\text{P}$ -chicken histone cDNA. DNA complementary to the 7–11S RNA sequences was synthesised with reverse transcriptase using random primers<sup>15</sup>. With 2  $\mu\text{g}$  of RNA and  $^{32}\text{P}$ -dATP of specific activity  $50 \text{ Ci mmol}^{-1}$ , we routinely obtained a yield of  $1.5 \times 10^7$  d.p.m. in cDNA. This product in  $2 \times \text{SSC}/0.5\%$  SDS was hybridised to a vast excess of 18S and 28S rRNA at  $60^\circ\text{C}$  to  $R_{0.1}$ . Ribosomal cDNA sequences were separated from putative histone cDNA sequences by sucrose gradient centrifugation (10–40% sucrose gradients centrifuged at 200,000g, 16 h at  $4^\circ\text{C}$ ). Fractions of 0.5 ml were collected from the gradient and  $^{32}\text{P}$ -radioactivity determined. The cDNA sedimenting at about 5S was pooled and used as a histone gene probe. ●,  $^{32}\text{P}$  c.p.m.; —,  $A_{254}$ .

on sucrose gradients (Fig. 3). About 30% of the cDNA sedimented with 18S and 28S rRNA, and the remaining 70% of the cDNA was present as a broad peak centred around 5S.

It was possible to test directly whether the 5S cDNA contained histone gene sequences. In a 'Southern' transfer experiment (Fig. 4), the 5S cDNA cross-hybridised to cloned sea urchin histone gene sequences (*Echinus esculentus* histone genes:  $\lambda$  clone 55, K. Murray and K. Peden, unpublished observations).

To test the proposal that this 5S cDNA contained predominantly histone coding sequences, the  $^{32}\text{P}$ -labelled cDNA was hybridised to total genome DNA from *E. esculentus*. *EcoRI*-digested sea urchin DNA was electrophoresed on agarose gels and transferred to nitrocellulose filters<sup>16</sup> (Fig. 5). It is possible to detect two closely migrating fragments (about 6 and 7 kilobases) which are identical in size to those detected by homologous *E. esculentus* histone gene probe. No other cross-hybridisation bands were detected when the chicken histone cDNA probe was used. (There are two separate size classes of histone gene repeats within the *E. esculentus* genome.  $\lambda$  Clone 55 contains the larger (7 kilobase) histone gene repeat unit (K. Murray,  $\lambda$  clone 55, K. Murray and K. Peden, unpublished observations).)

As discussed above, the most likely contaminants in the chick histone cDNA probe are ribosomal cDNA sequences. When cDNA probes from chicken rRNA were cross-hybridised to *EcoRI*-digested *E. esculentus* DNA (transferred to filters), the bands observed were readily distinguished from the characteristic 6 and 7 kilobase bands containing histone genes (Fig. 5c).

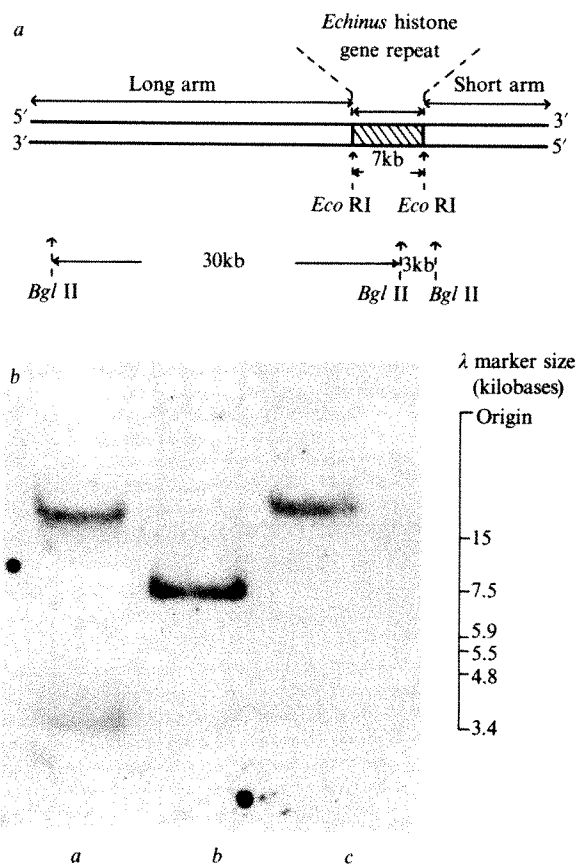
Therefore, we have shown that the 7–11S RNA prepared from chick embryos and passaged through poly(U)–Sephadex, programmes the synthesis of histones alone in an *in vitro* translation system. When cDNA is prepared from this RNA, and sequences complementary to rRNA are removed, the remaining cDNA can be used to detect sea urchin histone gene sequences. We have used this probe to detect histone genes in chicken DNA.

### The reiteration frequency of chicken histone genes

When chicken histone cDNA sequences were reannealed with genomic DNA from adult chickens, a single transition was

observed with a  $C_{0t_{1/2}}$  value of  $10^2$  (Fig. 6). This transition is distinct from that of unique sequences which have a  $C_{0t_{1/2}}$  value of  $10^3$ . These data show that histone genes are reiterated about 10-fold in the adult genome.

The rate of reannealing of histone genes is also quite distinct from that of ribosomal genes ( $C_{0t_{1/2}}$  value of 10 Fig. 6), and the data show that the chicken histone cDNA contained negligible amounts of ribosomal sequences.



**Fig. 4** Identification of chicken histone cDNA sequences, using cloned sea urchin histone genes. DNA from  $\lambda$  clone 55 (containing the 7-kilobase histone gene repeat from *E. esculentus*) was digested with *EcoRI* or *BglII*. Samples of 0.5  $\mu\text{g}$  were electrophoresed on 1% agarose gels (14 cm long, 2 mm thick, 1 cm lane width) at 60 mA for 4 h. DNA fragments were transferred to nitrocellulose<sup>16</sup>, baked in a vacuum oven for 3 h at  $80^\circ\text{C}$ , and soaked in Denhardt's solution<sup>17</sup> in  $2 \times \text{SSC}$  for 6 h at  $65^\circ\text{C}$ . Filters were dipped in  $^{32}\text{P}$ -labelled chicken histone probe (prepared as described in Fig. 3) in  $2 \times \text{SSC}/0.5\%$  SDS ( $10^6$  d.p.m. per  $\mu\text{g}$  cDNA). Strips were overlaid with paraffin oil and hybridisation allowed to proceed for 16 h at  $65^\circ\text{C}$ . After extensive washing in  $2 \times \text{SSC}/0.5\%$  SDS at  $65^\circ\text{C}$ , dry filters were put in contact with X-ray film for 2 days, and autoradiograms developed. The size of fragments were determined by reference to  $\lambda$  markers (cleaved with *HindIII* or *EcoRI*) run in the same gel, and detected by ethidium bromide staining. a, *EcoRI* and *BglII* restriction sites within  $\lambda$  clone 55. *EcoRI* excises the complete 7-kilobase histone gene insert and *BglII* cleaves the insert into two fragments. The sizes shown for the fragments are approximate. b,  $\lambda$  clone 55 restriction fragments hybridised to chicken histone cDNA and detected by autoradiography. a,  $\lambda$  clone 55 DNA restricted with *BglII*; b,  $\lambda$  clone 55 DNA restricted with *EcoRI*; c, unrestricted  $\lambda$  clone 55 DNA.

### Restriction endonuclease analysis of chicken histone genes

We have not been able to detect histone gene sequences in chicken DNA using a cloned sea urchin histone gene probe (K. Murray, K. Peden and J.R.E.W.). However, as shown in Fig. 5, the chicken cDNA probe can be used to detect *EcoRI* fragments



in sea urchin DNA of the expected size for the histone gene repeat. The reason for this apparent anomaly is probably related to gene numbers. Several hundred histone genes in sea urchin genomic DNA<sup>18</sup> could be detected by the chicken histone probe, but the 10 histone genes in the chicken genome were not detectable with the sea urchin probe. For all subsequent experiments involving restricted chicken DNA, we have used the homologous chicken probe.

Adult genomic DNA was prepared from chicken red blood cells<sup>20</sup> cleaved with the appropriate enzyme, and electrophoresed on agarose gels. After transfer of the DNA to nitrocellulose, the filters were impregnated with a solution containing <sup>32</sup>P-histone cDNA, and hybridisation was allowed to proceed at 65 °C for 16 h. Specific sequences were detected by autoradiography. Because of the sensitivity of the transfer procedure<sup>16</sup>, reiterated sequences such as ribosomal genes (100-fold reiterated in chicken, Fig. 6) are readily detected. We considered it necessary to determine the position of ribosomal gene fragments in endonuclease-restricted chicken DNA. In the event of trace contamination of histone cDNA with ribosomal cDNA sequences, ribosomal gene fragments could then be identified.

Tracks *b*, *d*, *f*, *h* in Fig. 7 show the location of ribosomal gene sequences on the same nitrocellulose filter previously used to detect histone genes. To achieve this, the hybridised <sup>32</sup>P-histone cDNA was allowed to decay sufficiently before the ribosomal cDNA probe was hybridised. Direct comparison could thus be made between the size of fragments containing the two different sets of genes within one transfer. For each of the four restriction enzyme experiments, histone genes are in different sized fragments of DNA compared with ribosomal genes.

Chicken histone cDNA was used to detect histone gene sequences in restriction endonuclease-cleaved chicken DNA. Fragments containing histone genes were detected in chicken DNA cleaved with *Bgl*II, *Eco*RI, *Bam*HI and *Hind*III (Fig. 7,

tracks *a*, *c*, *e* and *g*, respectively in Fig. 7). One outstanding feature in tracks *a*, *c* and *e* is the presence of a fragment about 15 kilobases long. We propose that this is the repeat length for the histone genes in the chicken genome.

Histone fragments generated by *Hind*III are shown in Fig. 7g. The additive size of the three *Hind*III histone DNA fragments (13–14 kilobases) is marginally less than the 15-kilobase repeat length seen consistently with *Eco*RI, *Bgl*II and *Bam*HI. One possible explanation is that a fourth histone fragment (between 1 and 2 kilobases long) is generated by *Hind*III cleavage. If the majority of this additional fragment was spacer DNA (sequences not represented in histone mRNAs), then the fragment would not be detected by the chicken histone gene probe.

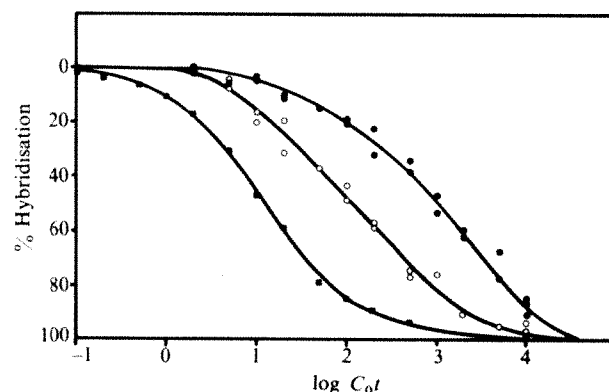


Fig. 6 Estimation of histone gene reiteration frequency in the chicken genome. Reassociation reactions of <sup>3</sup>H-labelled total chicken DNA (●) and of unlabelled genomic DNA in the presence of <sup>32</sup>P-labelled histone cDNA (○) or ribosomal cDNA (■) were set up and samples taken as described by Kemp<sup>19</sup>, except that 0.18 M NaCl was used in the hybridisation buffer. The degree of reassociation was assayed using nuclease S<sub>1</sub>. At the highest C<sub>0</sub>t values, the maximum level of hybridisation was about 70%; the data have been normalised to 100%.

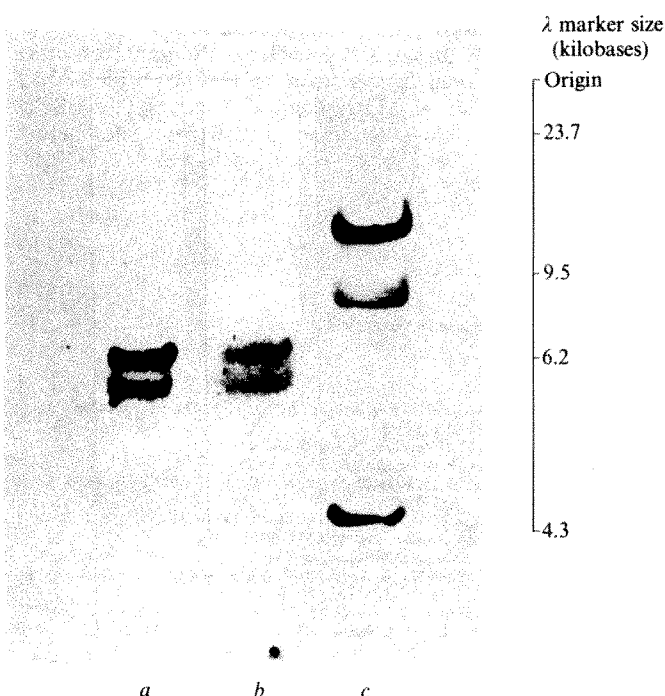
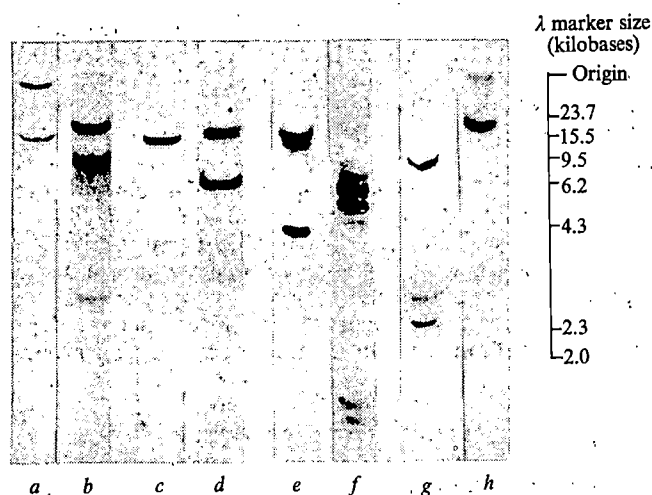


Fig. 5 Detection of 6- and 7-kilobase bands in sea urchin DNA, with chicken histone cDNA. Sea urchin DNA was digested to completion with *Eco*RI. Samples of 2 µg were electrophoresed on 1% agarose gels (30 cm long, 1 cm thick, 1 cm lane width) at 60 mA for 16 h. Fragments containing sea urchin histone genes were detected as described in Fig. 4. *a*, Bands (about 6 and 7 kilobases) detected with <sup>32</sup>P-*E. esculentus* histone gene probe. *b*, Bands in sea urchin DNA detected with chicken histone cDNA; *c*, Bands in sea urchin DNA detected with cDNA made on 18S and 28S chicken rRNA templates.

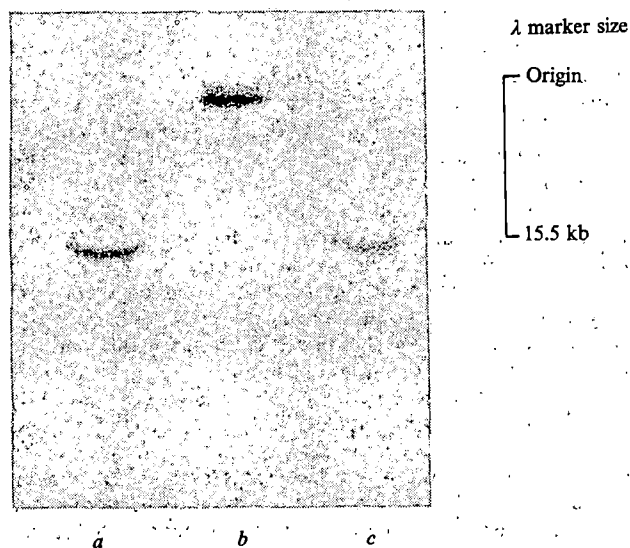
It is also apparent that both *Bgl*II and *Bam*HI digests reveal sequence heterogeneity in the histone genes. We have seen this heterogeneity in DNA prepared from single animals. In the case of the *Bam*HI histone gene pattern (Fig. 7e), two lower molecular weight bands are visible in addition to the 15-kilobase fragment. These two smaller bands (about 11.5 and 4.5 kilobases, respectively) together approximate to the size of the 15-kilobase repeat unit. They are not due to partial *Bam*HI cleavage, as there was no change in the histone gene pattern following further enzymatic digestion. This evidence suggests that there are two classes of histone gene repeats with respect to *Bam*HI cleavage sites; that is, one class contains one and the other class two restriction sites per histone gene repeat unit.

Two classes of histone gene sequences are also detected when chicken DNA is cleaved with *Bgl*II (Fig. 7a). One class is cleaved once per repeat to generate a 15-kilobase fragment; the other class does not contain restriction sites for *Bgl*II, as the high molecular weight DNA containing histone gene sequences hardly enters the gel. This material was not susceptible to further digestion with *Bgl*II. It is possible to separate the high molecular weight *Bgl*II histone gene fragment from the 15-kilobase *Bgl*II fragment by sucrose gradient centrifugation (data not shown). As indicated in Fig. 8 (track *c*), digestion of this high molecular weight *Bgl*II fragment with *Eco*RI gives rise to the unit histone gene repeat of 15 kilobases in the same way as does total chicken DNA when digested with *Eco*RI (Fig. 8a).

It is evident that there is some heterogeneity in the histone genes, and further, that precise details of chicken histone gene organisation require cloned fragments. However, given that restriction enzyme patterns show (1) a 15-kilobase histone unit alone, (2) a higher molecular weight histone fragment which can generate a 15-kilobase histone unit, or (3) histone gene bands



**Fig. 7** Detection of histone genes in chicken DNA cleaved with restriction enzymes. DNA isolated from chicken erythrocytes<sup>20</sup> was digested with restriction enzymes *EcoRI*, *BglII*, *BamHI* or *HindIII*. After phenol extraction and ethanol precipitation, the digested DNA (50 µg per track) was electrophoresed on 1% agarose gels, transferred to nitrocellulose<sup>16</sup> and hybridised with <sup>32</sup>P-labelled chicken histone cDNA, prepared as described in the legend to Fig. 3. After the histone fragments had been detected by autoradiography, the nitrocellulose strips were stored for 4 weeks to allow a significant level of decay of the <sup>32</sup>P-histone probe. The same nitrocellulose strips were then hybridised with <sup>32</sup>P-labelled cDNA synthesised from 18S and 28S chicken ribosomal RNA templates. The size of the fragments was determined using *HindIII*-cleaved λDNA and *Sall*-cleaved λDNA electrophoresed in the same gel as a standard. Lanes a, c, e and g were hybridised with chicken histone cDNA, and they contain chicken DNA digested with *BglII*, *EcoRI*, *BamHI* and *HindIII*, respectively. Lanes b, d, f and h were hybridised with chicken 18S and 28S ribosomal cDNAs, and they contain chicken DNA digested with *BglII*, *EcoRI*, *BamHI* and *HindIII*, respectively.



**Fig. 8** Digestion of the high molecular weight *BglII* histone gene fragment with *EcoRI*. Chicken DNA was digested to completion with *BglII* and centrifuged on 10–40% sucrose gradients in 0.01 M NaCl, 0.001 M EDTA, 0.01 M Tris-Cl, pH 7.5, at 200,000g at 4 °C for 16 h in a Beckman ultracentrifuge. The large *BglII* histone gene fragment was separated from the 15-kilobase *BglII* fragment (Fig. 7a) on fractionation of the gradient (data not shown). After *EcoRI* digestion of the DNA fraction containing the large *BglII* histone gene fragment, the DNA was electrophoresed on 1% agarose, transferred to nitrocellulose and hybridised with <sup>32</sup>P-chicken histone cDNA as described in the legend to Fig. 4. a, Histone gene sequences in total chicken DNA digested with *EcoRI*; b, large *BglII* chicken histone gene fragments fractionated by sucrose gradient centrifugation; c, Large *BglII* histone gene fragment in chicken DNA fractionated by sucrose gradient centrifugation and digested with *EcoRI*.

which add up to about 15 kilobases in size, we conclude that chicken histone genes are clustered and have a unit repeat length of 15 kilobases.

We believe this to be the first direct evidence for the histone gene repeat size in a higher eukaryote. It is not clear whether the 15-kilobase repeat contains genes coding for each of the five classes of histone proteins. The only histone gene repeats so far studied are those from sea urchin<sup>1–4</sup> and *Drosophila*<sup>5</sup>, and in these organisms at least, the histone repeat contains a complete set of histone genes.

More specifically, we have shown previously that the H5 genes (only expressed in erythroid cells) are reiterated 10-fold in the chicken genome<sup>21</sup>, and we show here that other histone genes are reiterated to the same extent. It is not clear whether H5 genes are contained within the 15-kilobase repeat. Nevertheless, there seems to be at least four to five times more DNA per repeat than is required for coding. It will be interesting to determine whether histone mRNAs are transcribed from one DNA strand (as in sea urchin<sup>3</sup>) or from both strands (as in *Drosophila*<sup>5</sup>). The report of a 15-kilobase RNA species containing histone sequences in HeLa cells<sup>22</sup> suggests the former possibility.

If 15 kilobases is representative of higher eukaryote histone gene repeat size, then this is about 2.5–3 times the length of its counterpart in sea urchin DNA and *Drosophila* DNA. If the arrangement of coding sequences and A/T-rich spacer sequences found in sea urchin histone genes<sup>4</sup> is similar in higher eukaryote histone genes, there would be a significant increase in length of 'spacer' DNA, as the length of coding regions for histones will be almost identical in all species. Nevertheless, the length of spacer DNA in a cistron cannot necessarily be equated with the evolutionary complexity of the organism. For example, the repeat length of ribosomal genes in *Dictyostelium* and mouse is comparable (38 (ref. 23) and 44 (ref. 24) kilobases, respectively) and both of these are much larger than the 11–14-kilobase repeat in *Xenopus*<sup>25</sup>. Whether the extra DNA in higher eukaryote histone genes exists as intervening sequence DNA within coding regions or as elongated spacer regions between histone coding regions, remains to be determined.

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# letters

## Production of new cosmological perturbations during the radiation-dominated era

PURCELL (unpublished data) has noted the tendency for 'dust' particles strongly to cluster when they are coupled by viscous drag to a 'gas' whose velocity field is stochastic:

$$\frac{dV_{\text{dust}}}{dt} = \alpha \{V_{\text{gas}}(x, t) - V_{\text{dust}}\} \quad (1)$$

where  $\alpha$  ( $\text{s}^{-1}$ ) is the viscous coupling constant. The application of the Purcell clustering effect to cosmology which is considered here generates relatively large isothermal perturbations from relatively small adiabatic perturbations which will not otherwise survive damping during recombination.

The Purcell clustering effect seems at first to be paradoxical as one would expect a stochastic gas flow to randomise, rather than cluster, the positions of independent, non-interacting dust particles. The explanation is that the velocity field is not completely random but consists of disturbances that propagate at sound velocity. Therefore, on a given scale the gas velocity will be correlated for one sound travel time. In linear order in the gas velocity the dust particles always return to their original positions after the passage of an acoustic wave; in quadratic order, however, transport effects are present<sup>1,2</sup>. The accelera-

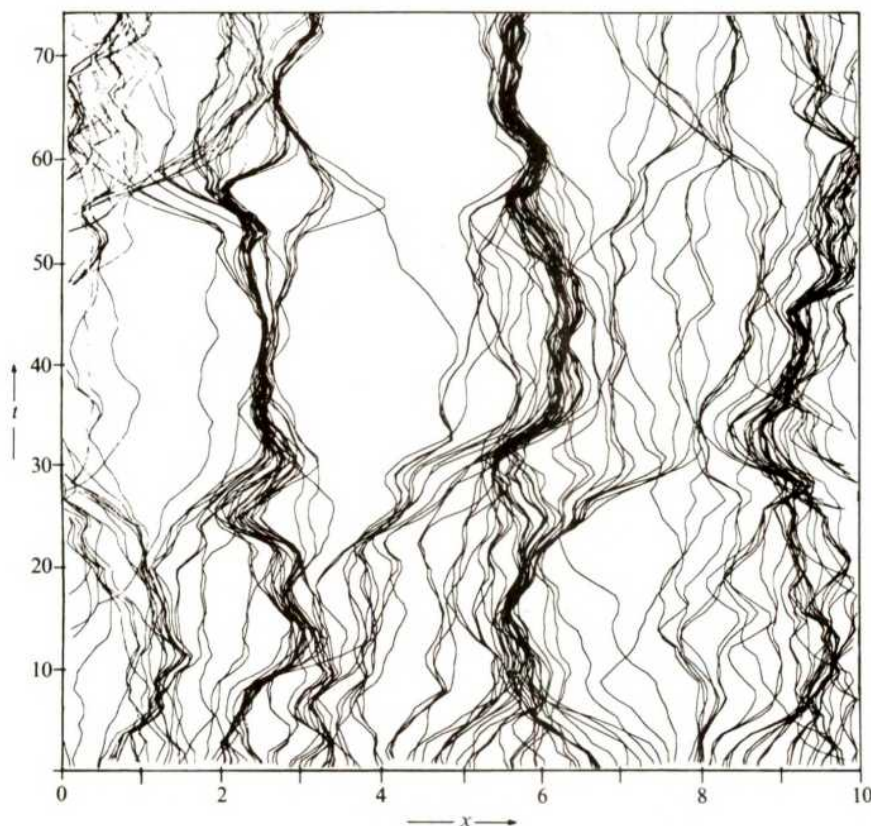
tion in quadratic order on a given scale will go as  $V_{\text{gas}}^2/l$ . In the low frequency limit, that is, for scales  $l \gg c_s \alpha^{-1}$ , particles are strongly coupled (frozen in) to the gas. The particles will therefore acquire a velocity relative to the gas of  $u \sim V_{\text{g}}^2/\alpha l$ . The mean square clustering of particles due to this anomalous motion per sound travel time is  $\delta^2 \sim (u/c_s)^2$ . The clustering of the particles at later times will not be correlated with the original clustering and will add randomly in the mean square sense. The growth of particle clustering with time will therefore be

$$\left(\frac{\delta\rho}{\rho}\right)_t^2 \sim \left(\frac{V_{\text{gas}}}{c_s}\right)^4 \left(\frac{c_s}{l\alpha}\right)^3 \alpha t \quad (2)$$

for  $l \geq c_s \alpha^{-1}$ . On small scales  $l \ll c_s \alpha^{-1}$ , the particles will always be in the process of relaxing towards the velocity  $u$  given above. The typical response to the quadratic term will be  $(1 - e^{-\alpha/\omega})u$  where  $\omega$  is the frequency corresponding to the scale  $l$ . The anomalous motion of the particles relative to the gas will therefore be  $V_{\text{g}}^2(\alpha/\omega^2)$ . In the same manner we may see that in the high frequency limit the particle clustering is given by

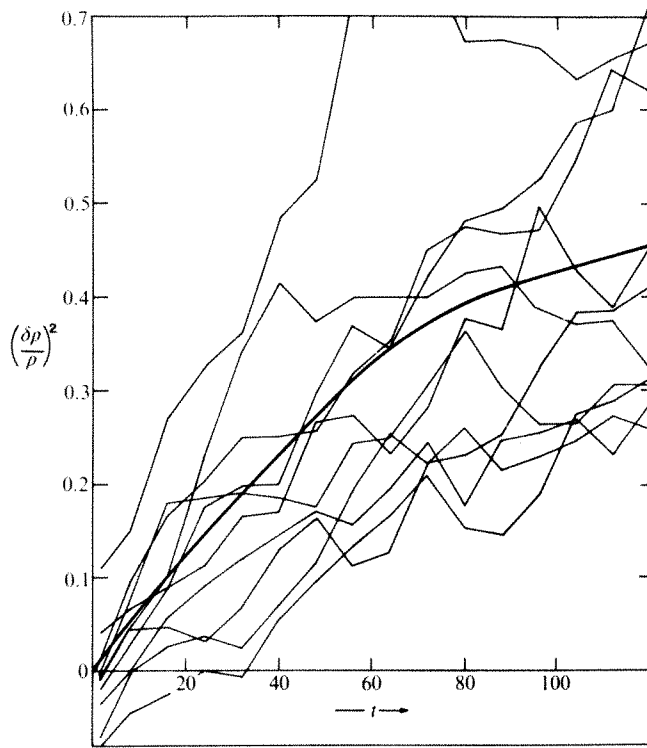
$$\left(\frac{\delta\rho}{\rho}\right)_t^2 \sim \left(\frac{V_{\text{gas}}}{c_s}\right)^4 \left(\frac{\alpha}{\omega}\right) \alpha t \quad (3)$$

We will give a detailed analytic derivation of the above equations elsewhere. Without considering the above physical picture the ensemble-average mean square increase of clustering can be computed using time from a formal expansion of equation (1)



**Fig. 1** Space-time diagram showing the aggregation of particles by a random acoustic field. First order motion, which integrates to zero over time, has been removed from the picture. Gaussian acoustic white noise propagates both leftwards and rightwards at velocity  $dx/dt = 1$ . The viscous coupling time of particles to the gas is  $\alpha^{-1} \approx 2$   $t$ -units, so clustering is primarily on a scale of about 2  $x$ -units. In the period shown, about 40 uncorrelated small consolidations add in the mean-square sense to give a density perturbation of order unity.





**Fig. 2** Results from many runs as in Fig. 1. The squared clustering measure  $(\delta\rho/\rho)^2$  evaluated at a spatial scale of 2  $x$ -units is shown plotted against time. Narrow lines are 10 individual runs; the heavy line is the mean of 30 further runs. For  $(\delta\rho/\rho)^2 \leq 0.3$ , squared clustering varies linearly with time, as predicted by the analytic theory. For larger  $(\delta\rho/\rho)^2$  deviations from linear growth become visible, due to numerical brownian motion on the differencing scale.

and the equation of continuity. Here we summarise the results of numerical experiments which corroborate the analytic derivations, and with an immediate application to cosmology.

An example of a numerical experiment in one dimension is shown in Fig. 1, where 128 particles are shown clumping to order unity density contrast on a scale containing about 25 particles. In this experiment density contrast was measured at the scale where  $\omega \sim \alpha$ . (Only the scale, not the number of particles shown, is relevant: 12,800 particles in the same hydrodynamic velocity field would have clustered on scales containing 2500 particles.)

Figure 2 shows  $\delta\rho/\rho$  as a function of time for 10 runs which differ only in having different random sequences of sound waves. Also shown is the mean of 30 additional runs. The secular nature of the clustering effect is evident. In equation (2), the value of the coefficient taken from the experiments agrees with the value computed analytically to within 20%. In three dimensions, equation (2) also holds, with a somewhat different numerical coefficient. The coefficient is weakly dependent on the slope of the power spectrum of the adiabatic perturbations.

We now consider the application of the Purcell clustering effect to cosmology, namely its ability to generate relatively large isothermal perturbations (which can make globular clusters and perhaps galaxies in the post-recombination era) from relatively small adiabatic perturbations which will not otherwise survive damping during recombination. Purcell clustering is able to generate these perturbations on scales much smaller than the Jeans mass, during the radiation dominated era ( $z > 10^3$ ) when, according to current views, matter and radiation are tightly coupled.

Weinberg<sup>3</sup> and others have recently pointed out that baryon non-conserving reactions at high energies (a consequence of the

charge-conjugation parity (CP) violation in super-unified theories) will 'cook' the early Universe to a unique value of entropy per baryon, one which in particular cannot vary from point to point spatially. Therefore late-time processes like Purcell clustering (or shock dissipation, see below) may well be the only way to introduce isothermal (entropy) perturbations into a cosmology.

In our cosmological application, the dust particles of Purcell clustering are individual protons and helium nuclei of the pre-recombination, radiation dominated, Friedmann cosmology; the dominant component of the gas is photons; the ionised plasma (dynamically insignificant) supplies the necessary scattering opacity to render the mean free path of photons much shorter than any other scale of interest. However, the finite mean free path of the photons will cause acoustic waves to damp long before the time when  $k = \alpha c_s^{-1}$  [ref. 4].

The baryons are viscously coupled to the photon gas primarily by radiation drag on their attendant electrons<sup>5</sup>, with

$$\alpha = \frac{4\sigma_T a T^4}{3m_p c} = 3.1 \times 10^{-11} \text{ s}^{-1} \left( \frac{z}{1300} \right)^4 \quad (4)$$

where  $\sigma_T$  is the Thompson cross section,  $m_p$  the proton mass, and  $z$  is the redshift of the epoch considered. The temperature now is taken to be 3 K. The Hubble time  $t_h$  as a function of  $z$ , when the Universe was photon-radiation dominated<sup>6</sup>, is

$$t_h = a/\dot{a} = \left( \frac{8\pi}{3} G\rho \right)^{-1/2} = 3.0 \times 10^{13} \text{ s} \left( \frac{1300}{z} \right)^2 \quad (5)$$

So,

$$\alpha t_h = 940(z/1300)^2 \quad (6)$$

The sound speed in the radiation dominated era is  $c_s = c/3^{1/2}$ , so the characteristic baryon rest mass contained in a volume of characteristic size  $c_s/\alpha$  is

$$M_P = \frac{4\pi}{3} \rho_m \left( \frac{\pi c_s}{\alpha} \right)^3 = 1.7 \times 10^{10} M_\odot \left( \frac{1300}{z} \right)^9 \left( \frac{\Omega}{0.1} \right) \left( \frac{H_0}{60} \right)^2 \quad (7)$$

If we substitute equations (7) and (6) into equation (2) we obtain

$$\left( \frac{\delta\rho}{\rho} \right)_{\text{iso}} \sim 310 \left( \frac{\delta\rho}{\rho} \right)_{\text{ad}}^2 \left[ \left( \frac{\Omega}{0.1} \right) \left( \frac{H_0}{60} \right)^2 \right]^{1/2} \quad (8)$$

where we have taken a coefficient of 10 in equation (2) from more exact calculations. Equation (8) is valid only for  $M > M_c$ , the damping scale at recombination. We note that  $M_c = 9.8 \times 10^{12} [(\Omega/0.1)(H_0/60)^2]^{1/2} M_\odot$  [ref. 7]. For  $M < M_c$  the waves driving the clustering are damped before recombination. As  $(M/M_c) = (t/t_h)^{9/4}$  gives the damping time for mass scales  $< M_c$  we get

$$\left( \frac{\delta\rho}{\rho} \right)_{\text{iso}} \sim 2 \left( \frac{\delta\rho}{\rho} \right)_{\text{ad}}^2 \left( \frac{M}{M_P} \right)^{5/18} \left[ \left( \frac{\Omega}{0.1} \right) \left( \frac{H_0}{60} \right)^2 \right]^{7/6} \quad (9)$$

Equations (8) and (9) say, roughly, that 10% adiabatic perturbations will generate 1% isothermal perturbations anywhere in the mass range  $10^9$ – $10^{12} M_\odot$ .

In the 'pancake' theory of galaxy formation as originally proposed by Zel'dovich, Sunyaev *et al.*<sup>8–11</sup>, the density contrast before recombination is the same at all scales with a value between  $10^{-3}$  and  $10^{-4}$ . In this case Purcell clustering is insignificant. However, calculations by Silk<sup>12</sup> and by Peebles and Uy<sup>13</sup> seem to show that a considerably larger density contrast on the scale of the pancakes is necessary to produce galaxies, say  $10^{-2}$ . As limits of  $< 10^{-3}$  on the large-scale inhomogeneity can be set from the microwave background<sup>14</sup>, it seems likely that a viable perturbation spectrum must have  $\delta\rho/\rho$  increasing towards smaller masses and reaching the 10% level well within the mass range where Purcell clustering becomes important. In that case the condensation of sub-galactic units renders the subsequent collapse of the pancake stellar dynamical rather than hydrodynamical. The formation of dense, cold sheets needed for fragmentation into galaxies is impossible, and the whole pancake hypothesis must be reexamined.

The Purcell clustering process is physically distinct from another process which can generate entropy fluctuations, namely the dissipation of shock waves that are formed by wave steepening, and wave-wave interactions. For 10% adiabatic fluctuations described above, the Purcell clustering seems to dominate for two reasons: first incoherent acoustical disturbances steepen much more slowly than plane waves<sup>15</sup>, and second shock dissipation goes as the cube of shock strength, so weak (10%) shocks are not very dissipative.

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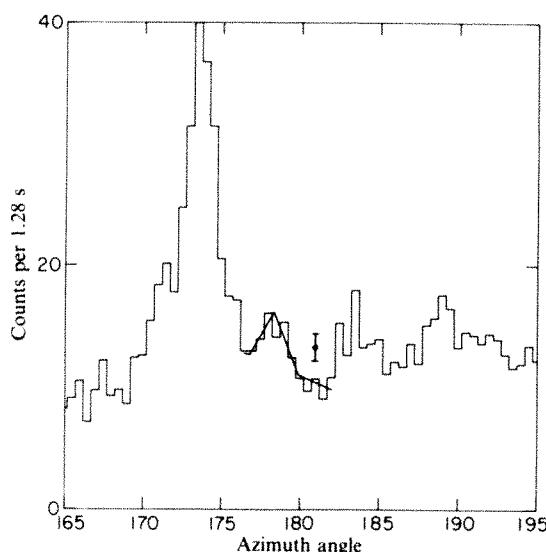
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## Evidence for X-ray emission from Kepler's supernova remnant

THE remnant of Kepler's supernova of 1604 AD has long eluded detection at X-ray wavelengths, principally because of its proximity to a source confused region in the direction of the galactic centre. We present here the first evidence for weak soft X-ray emission from Kepler's remnant, which was beyond the capability of earlier X-ray surveys<sup>1</sup> but is now possible because of the increased sensitivity provided by the low energy detectors (LEDs) of the A 2 experiment on the HEAO 1 spacecraft. (The A 2 experiment on HEAO 1 is a collaborative effort led by E. Boldt of GSFC and G. Garmire of CIT, with collaborators at GSFC, CIT, JPL, and UCB). The LEDs are described in detail by Rothschild *et al.*<sup>2</sup> Briefly, the data reported here were acquired using the LED1 narrow field of view (1.5° FWHM in the scan direction) which has an effective area of 175 cm<sup>2</sup> and is sensitive to X-rays between 0.2 and 3 keV.

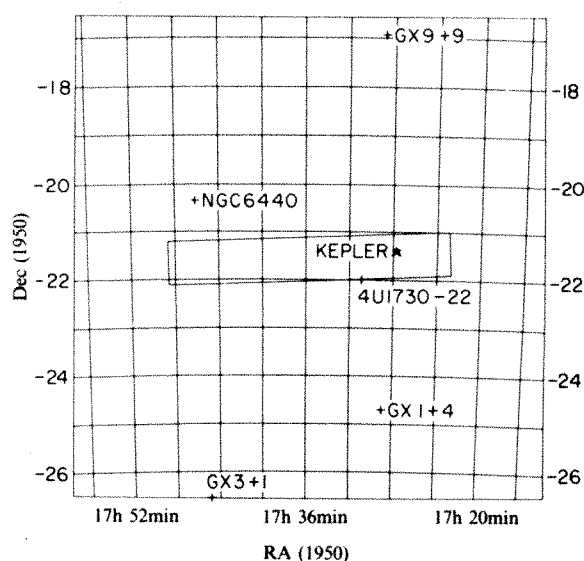
The region of sky containing Kepler's remnant was scanned by LED1 between 11 and 16 March 1978, and Fig. 1 shows the results of superposing five scans during this time interval. The count-rate data are complex owing to the concentration of X-ray sources in the direction of the galactic centre. However, a new soft source, denoted H1735-21, is evident in the data as a count-rate peak centred at a scan angle of 178.2°. This is just resolved from the GX9+9 region. The solid line in Fig. 1 indicates a least-squares fit of the LED1 angular response function to the data bounded by the sources GX9+9 and GX3+1. The 90% confidence error box derived from this fit is depicted in Fig. 2, together with the positions of all known nearby X-ray sources. Incorrect modelling of the background could introduce small systematic error (~0.1°) in position of narrow direction of the error box. Kepler's remnant lies within our error box, and is therefore proposed as the probable identification. We have checked other classes of object which could produce soft X-ray emission, but no other compelling counterparts were found within the error box. The Uhuru source 4U1730-22 (ref. 3), which lies at the edge of the 90% confidence error box, is an unlikely identification because it is



**Fig. 1** A superposition of five scans of HEAO A-2 LED data (0.3-3.0 keV). A typical error bar around the region of interest is shown. The solid curve represents the best fit to the triangular response of our detectors to H1735-21 plus the local background. GX9+9 (angle = ~174) and GX3+1 (angle = ~183) constrained the use of background data.

known to be a transient<sup>4</sup>. The spectrum of transients are hard near maximum intensity. The absence of H1735-21 in medium and high energy detectors (S. Pravdo, personal communication) precludes it being the transient detected during an 'on-state'. Our data are consistent with the source being constant during the period of observation. However, because the source is just at our level of detectability, we are insensitive to decreases in intensity.

The distance to Kepler's remnant remains uncertain. Because it lies in the direction of the galactic centre the application of normal distance measuring techniques (for example, neutral hydrogen absorption observations) is impossible. A position within the nuclear bulge, with distance of about 10 kpc, has been invoked to help explain the comparatively large angular displacement from the galactic plane. The historically inferred apparent magnitude of -3, and its classification as Type I (ref. 5), suggest a distance of between 3.5 and 6 kpc, assuming an



**Fig. 2** A map of the region shows the derived HEAO A-2 90% confidence error box. The position of Kepler's SNR as well as all known nearby X-ray sources is shown. Note the position of 4U1730-22 is at the edge of the box.



absolute magnitude for a Type I supernova of  $-19$  and visual absorption in the range  $0.5$  to  $1$  mag  $\text{kpc}^{-1}$ . Recent unpublished observations of the reddening of the optical remnant of Kepler's supernova (I. J. Danziger, personal communication) suggest a distance of  $\sim 4$  kpc.

The intensity derived for H1735-21 is  $(6.3 \pm 1.5) \times 10^{-2}$  counts  $\text{cm}^{-2} \text{s}^{-1}$  between  $0.3$  and  $3.0$  keV, corresponding to a flux of  $(2.4 \pm 0.6) \times 10^{-10}$  erg  $\text{cm}^{-2} \text{s}^{-1}$ , assuming a power law spectrum with a photon index of  $-2.0$  and a column density of  $10^{22}$  H-atoms  $\text{cm}^{-2}$ . (The count flux as well as the energy flux could be uncertain by a factor of 2 due to uncertainties in background subtraction and incident spectrum.) The assumed hydrogen column density, probably appropriate for Kepler's remnant regardless of distance uncertainty, gives an intrinsic X-ray luminosity in the energy range  $0.3$ – $3$  keV of  $\sim 180 \times 10^{35}$  erg  $\text{s}^{-1}$  for a distance of  $10$  kpc, reducing to  $\sim 28 \times 10^{35}$  erg  $\text{s}^{-1}$  for a distance of  $4$  kpc. The former value is greater than for any other known young supernova remnant, including the Crab Nebula, and must cast further doubt on Kepler's remnant lying at the galactic centre distance. The latter value is more acceptable, being comparable with that of other young supernova remnants such as that for Tycho's SNR and Cas A.

On the near side of the Galaxy, six supernovae recorded in historical times have been identified with remnants<sup>5</sup>—those of 185 AD (remnant RCW 86), 1006 AD (PKS 1459-41), 1054 AD (the Crab Nebula), 1181 AD (3C 58), 1572 AD (Tycho) and 1604 AD (Kepler). The objects Cas A, RCW 103 (ref. 6), and MSH 11-54 (refs 7, 8) are also believed to be the remnants of supernovae which have occurred within the past two millennia but were not recorded historically. All the above mentioned objects have previously been detected in X-rays with the exception of Kepler's remnant. For this reason, it has long been classified as a highly likely X-ray source<sup>9,10</sup>, although problems of confusion and sensitivity have thwarted previous attempts at detection. HEAO 2 will now allow the detection reported here to be confirmed, the source to be imaged, and its spectrum to be more accurately determined.

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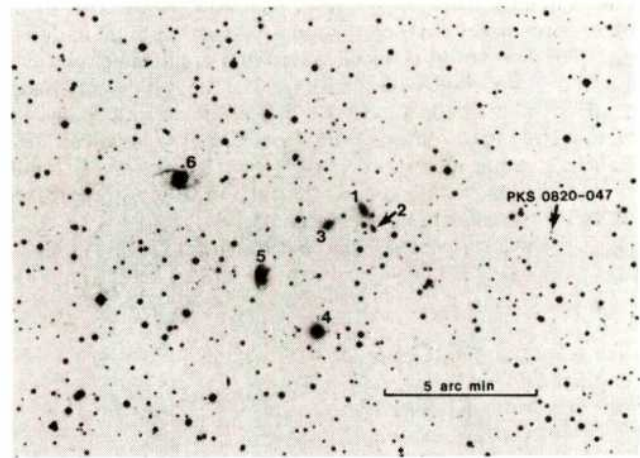
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## A peculiar galaxy system in Hydra

IN the course of scanning the Palomar Sky Survey, I noticed what appeared to be a large and a small elliptical galaxy interconnected by a long straight bridge which could have been a radial jet from the larger galaxy. Furthermore a comparison between red and blue prints showed the smaller galaxy to be



**Fig. 1** The cluster of galaxies containing the peculiar system (from the blue print of the Palomar Sky Survey). Identifications of galaxies are as follows: the peculiar system (galaxy 1 = MCG -1-22-6 = F-272; galaxy 2 = F-271; galaxy 3 = MCG -1-22-7 = F-273). Galaxy 4 = NGC2583 = MCG -1-22-8; galaxy 5 = MCG -1-22-9; galaxy 6 = MCG -1-22-10. The optical identification of the radio source PKS0820-047 is also indicated. (Reproduced with permission from the National Geographic Palomar Observatory Sky Survey.)

extremely blue, and thus highly unusual. Detailed enlargement of the images suggest that the larger galaxy may possess chaotic filaments akin to those in M82 or NGC1275. The spectroscopic observations described here show that, as expected, the two galaxies have strong narrow emission lines, but also that a third neighbouring galaxy is involved.

The system concerned appears as part of a medium-poor cluster of galaxies at  $1950: 8^{\text{h}} 20.7^{\text{m}}, -4^{\circ} 50'$  in the constellation of Hydra (see Fig. 1). The two larger members of the peculiar system are recorded in Vorontsov-Velyaminov's *Morphological Catalogue of Galaxies*<sup>1</sup> (MCG) although the system is apparently not included in his *Atlas of Interacting Galaxies*<sup>2</sup>. (The designations F-271, F-272 and F-273 refer to the selection of these objects for my current survey of 'compact and bright-nucleus galaxies'<sup>3</sup>.)

Figure 2 shows further enlargements of the peculiar system. These plates are made from a different copy of the Palomar Sky Survey from that on which the pair of ellipticals were originally noticed and the straight bridge between those galaxies (galaxies 1 and 2) is, in this case, not as conspicuous as it appeared on the red print of the first copy. This seems to be why Vorontsov-Velyaminov<sup>2</sup> did not pick it up; had he done so it would have made an extreme example of his 'bottle form' category.

The extreme blue colour of galaxy 2 is readily apparent by comparing its images in Fig. 2a and b; similarly, the red filaments of galaxy 1 (the appearance of the outer parts of the galaxy is slightly affected by the mottled sky background of the paper print). Galaxy 3 seems to possess bluish outer regions and condensations.

Spectroscopy was carried out in 1978 November/December with the image tube spectrograph on the 1.9 m Radcliffe Reflector at the South African Astronomical Observatory. Unwidened spectra at  $210 \text{ \AA mm}^{-1}$  covering  $3,700$ – $7,000 \text{ \AA}$  with a strong peak in the blue were obtained (and reduced in the same manner as described elsewhere<sup>3</sup>). The spectrum of galaxy 1 shows strong [O III]5007+4959 as well as narrow H $\beta$ ; the emission region seems more extensive than the central core of the galaxy (it could be filaments). The uncorrected redshift is found to be  $z = 0.0218$  (probable error 0.0005). The spectrum of galaxy 2 reveals strong narrow lines of [O II]3727, H $\beta$ , [O III]5007+4959 and H $\alpha$ , with  $z = 0.0230$ . Unexpectedly, galaxy 3 also turned out to have emission lines though they are weaker; it has narrow [O II]3727, H $\beta$  and [O III]5007+4959 with  $z = 0.0226$ . NGC2583 (galaxy 4) seems to have a con-



ventional absorption spectrum with  $z=0.0197$  (based on the G-band and H and K lines, with a slight uncertainty in identification). Galaxies 5 and 6 proved to be of low surface brightness and identification of features in their spectra proved very difficult; both may have very weak H $\beta$  emission (as might be expected for such late type spirals) with  $z=0.0240$ . (As all six galaxies are relatively faint, the spectrograms show appreciable sky contamination. Thus the overall quality does not permit quantitative measurements of line strengths and so on).

The strong narrow emission lines of galaxies 1, 2 and 3 indicate abnormal nebular emission—as is not uncommon in interacting systems (such as, nos 44–45–46, 156–157, 199, 204 and 210 in my current survey<sup>3</sup>). The astrophysical interpretation of such is usually controversial. In this case it looks as though galaxy 2 has either been ejected from galaxy 1, or at least has passed through it. If the latter is the case, the tidal turmoil has evoked a sudden wave of star formation providing the excitation mechanism for the nebular emission. But are the red filaments of galaxy 1 merely tidal in origin, and how does galaxy 3 fit into such a picture? The alternative would be in line with ideas favoured by Arp<sup>4</sup>—that galaxy 1 is some sort of active galaxy and galaxy 2 has somehow been thrown out from it—red

filaments would support ejective or explosive processes. The notion of an exploding galaxy immediately brings to mind M82 and one may question whether this is really an explosion or are the active trio merely a manifestation of galaxies drifting through dust<sup>5</sup>? None of these explanations seem satisfactory and further speculation should probably await the photography and spectroscopy of this system by larger telescopes.

Figure 2 also indicates some particularly red and blue stars; it is a little unusual that they should appear to lie so close to the galaxies but there is nothing to suggest that they are not foreground stars. Likewise the radio source PKS0820-047<sup>6</sup> (= OJ-033=4C-4.26) identified with an 18th magnitude starlike object lies close enough to appear in Fig. 1.

I am grateful for the hospitality at the A. J. Dyer Observatory of Vanderbilt University, Nashville, Tennessee (where this object was originally noticed in early 1977) and to the director of the South African Astronomical Observatory for observing time.

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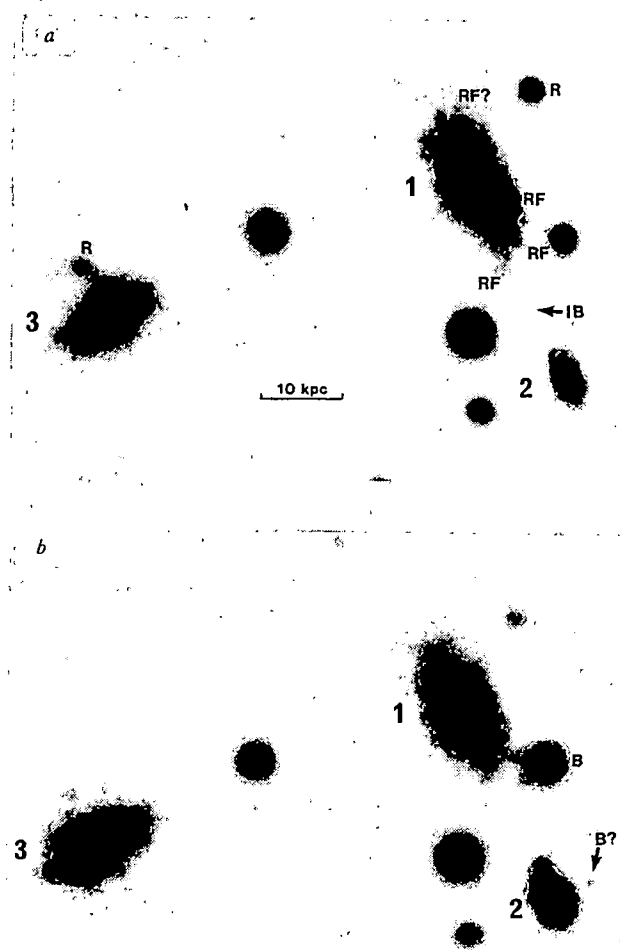


Fig. 2 Enlargements of the peculiar system from the red (a) and blue (b) prints of the Palomar Sky Survey; on a different copy of the survey galaxies 1 and 2 appeared interconnected by a straight red bridge—see text: IB, position of interconnecting bridge; RF, red filaments; R, particularly red stars; B, particularly blue stars. The scale is based on  $H=50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ . (Reproduced with permission from the National Geographic Palomar Observatory Sky Survey).

## A measurement of the Rydberg constant

THE Rydberg constant is of key importance in spectroscopy and, because it may be expressed as the product of several fundamental physical constants, it also plays an important part as an auxiliary constant in the evaluation of the fundamental constants of physics<sup>1</sup>. We report here a value of the Rydberg constant that is derived from the measurement of three of the fine-structure components of the hydrogen Balmer- $\alpha$  line by the saturable absorption technique<sup>2</sup>. The earlier measurements of the Rydberg constant by conventional spectroscopy and by the laser saturation absorption spectroscopy technique have been reviewed by Series<sup>3,4</sup>.

A c.w. tunable dye-laser (CR-599), pumped by the 514 nm line of an argon-ion laser (SP-170), was used as the source of monochromatic radiation. The dye-laser beam was split into counter-propagating saturating ( $\sim 10 \text{ mW}$ ) and probe beams ( $\sim 4\%$  of the saturating power), about 2 mm in diameter, these crossed at a small angle, about 1 mrad, at the centre of a Wood's discharge tube which contained hydrogen gas and residual water vapour. The central portion of the tube was 20 mm in diameter and 250 mm long. The voltage gradient, for a discharge current of 30 mA, at a hydrogen pressure of about 6 Pa (0.05 torr) was  $0.5 \text{ V mm}^{-1}$ . The dye-laser was adjusted to operate at the Balmer- $\alpha$  wavelength by making rapid measurements with a digital wavemeter<sup>5</sup>. The wavelength of the radiation was measured with a plane Fabry-Perot interferometer<sup>6</sup>, whose plates were silvered to give a finesse of about 35. The length of the etalon was about 97.2 mm and was stabilised to be an integral number of half-wavelengths of the radiation from a helium-neon laser which was locked to one of the fine-structure components of  $^{127}\text{I}$ . The wavelengths of these components are accurately known in terms of the  $^{86}\text{Kr}$  definition of the metre.

**Table 1** Values obtained for the reciprocal wavelengths of the fine-structure components and the random, systematic and total uncertainties in the last digit quoted

Fine-structure component	Wavenumber (m <sup>-1</sup> )	Uncertainties (s.d.)			No. of observations
		Random	Systematic	Total	
2P <sub>3/2</sub> -3D <sub>5/2</sub>	1,523,307.0507	30	104	108	39
2S <sub>1/2</sub> -3P <sub>3/2</sub>	1,523,336.5129	61*	135	148	13
2P <sub>1/2</sub> -3D <sub>3/2</sub>	1,523,340.0281	65*	130	145	13

\* These random errors are correlated

Signals from a confocal Fabry-Perot interferometer (125 MHz fsr) and the plane Fabry-Perot interferometer, together with the saturated absorption signal, were digitised and recorded. A computer was used to fit fringe intensity curves to the etalon signals, and also to fit combined lorentzian and gaussian distributions to the saturated absorption signal. Corrections were made to the measured wavelengths in order to allow for the phase-shift at the etalon mirrors<sup>6</sup> (-2.6 p.p.b., that is -2.6 parts in 10<sup>9</sup>), Stark shifts<sup>7</sup> (4.6 p.p.b., -3.3 p.p.b., and 1.3 p.p.b.), a correction for a possible pressure shift (4.4 p.p.b.) and for the unresolved cross-over signals<sup>8</sup> (-4.2 p.p.b., -8.4 p.p.b. and -13.8 p.p.b.), where the corrections for the three components have (where different) been given in the same order as in Table 1. The results were investigated for possible systematic dependence on the discharge conditions and laser power and, although no significant effects were found, an allowance was made for possible dependence on these parameters in making the systematic error assignments. The width and the asymmetry (which averaged to zero) of the resonances were also studied<sup>9</sup>.

**Table 2** Values obtained for the Rydberg constant since 1969

	Rydberg constant less 10,973,730 m <sup>-1</sup>	Standard deviation uncertainty (10 <sup>-3</sup> m <sup>-1</sup> )
Taylor <i>et al.</i> (1969) (review value)	1.20	1,100
Masui (1971)	1.88	800
Kessler (1973)	2.08	850
Kibble <i>et al.</i> (1973)	2.60	800
Hansch <i>et al.</i> (1974)	1.43	100
Goldsmith <i>et al.</i> (1978)	1.476	32
Present work	1.513	85

The values obtained for the wavenumbers of the three fine-structure components studied are given in Table 1, together with the random and systematic uncertainties (expressed as standard deviations) of the last digit quoted. These three values were ratioed with those calculated by Erickson (ref. 10 and personal communication) and then combined with his assumed Rydberg value to give a weighted mean value of 10,973,731.513(85) m<sup>-1</sup> for the Rydberg constant; our three Rydberg values agreed to 0.003 p.p.m. The standard deviation uncertainty assigned to our Rydberg measurement consists of the random uncertainty of the mean of 0.0021 m<sup>-1</sup> (55 independent results) which has been combined with the root sum square of the systematic uncertainties, expressed as a standard deviation, of 0.0082 m<sup>-1</sup>. Our result is in excellent agreement with other measurements of the Rydberg constant that have been made during the past 10 years<sup>8,11-15</sup>, (see Table 2 and ref. 4), particularly the saturable absorption measurement of Hansch *et al.*<sup>8</sup> and the laser polarisation spectroscopy measurement of Goldsmith *et al.*<sup>15</sup>.

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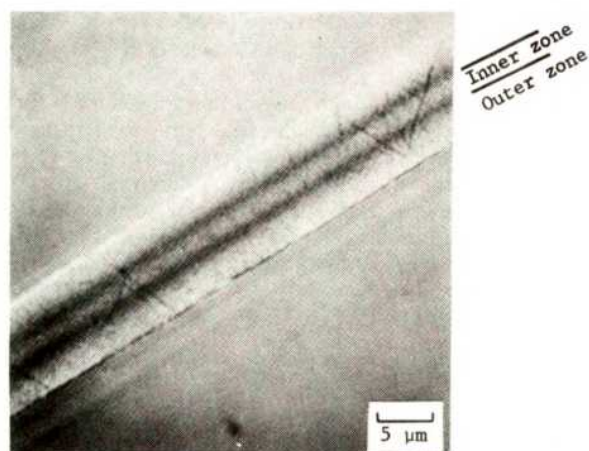
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## Optical anisotropy of carbon fibres

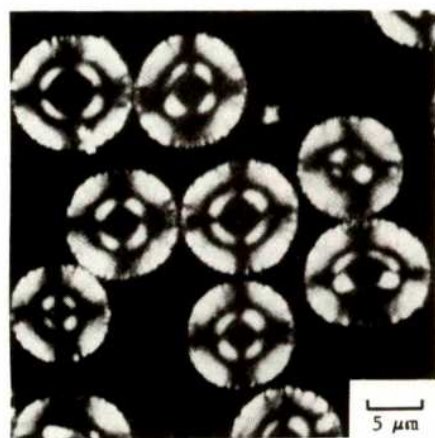
TECHNIQUES based on the interference of polarised light have been used to investigate double refraction in transparent materials due to the structural anisotropy of crystalline solids and strain birefringence in amorphous solids<sup>1</sup>. Forrest and Marsh have discussed<sup>2,3</sup> reflection methods and have used them to examine the anisotropy of the opaque solids carbons and cokes. As graphite is a uniaxial crystal with an optical c-axis perpendicular to the layer planes, polarised light incident on the layer-planes is not affected so that a polished section with this orientation appears isotropic. However, polarised light reflected by the prismatic faces is rotated and a polished section in this orientation shows optical anisotropy. By inserting a one wavelength retarder plate at 45° in front of the analyser of a polarising reflection-microscope with crossed polariser and analyser, interference colours are produced from which the orientation of the surface crystallites can be determined.

Polished cross-sections of carbon fibres made from mesophase pitch and examined in polarised light have a narrow 'Maltese-Cross' pattern with interference colours, deep yellow and blue, in alternate quadrants. By using pyrolytic graphite as a reference material, it can be inferred from the colours that the layer planes are arranged radially in the cross-section. Thin cross-sections which were cut and examined in a transmission electron microscope have confirmed this structure.

High modulus carbon fibres made by the RAE process<sup>4</sup> from textile PAN fibres (a polyacrylonitrile copolymer) also show a 'Maltese-Cross' but the pattern and the interference colours depend on the diameter of the PAN precursor fibre and the conditions used to convert it to a carbon fibre. The PAN fibres are first oxidised<sup>4</sup> under tension at temperatures in the range 200-300 °C. At the lower temperature, about 200 °C, the rate of chemical reaction is slow and the rate of diffusion is sufficiently fast to prevent a significant oxygen gradient. At higher temperatures, ≥220 °C, the reaction rate increases so that the oxidation becomes diffusion controlled and if oxidation is incomplete there is<sup>5,6</sup> a core with little oxidation surrounded by a more oxidised sheath. Longitudinal section (Fig. 1a) of a carbon fibre made after incomplete oxidation shows two zones and cross-sections (Fig. 1b) show two concentric interference figures separated by a dark (isotropic) ring, and having a centre which is also isotropic. From the interference colours these patterns have usually been interpreted<sup>7</sup> as an inner zone of radially orientated



a



b

**Fig. 1** Polished sections of carbon fibres made from 3 denier polyacrylonitrile copolymer fibre by heating in air for 6 h at 220 °C, then carbonising and heating to 2,500 °C. Polariser and analyser crossed. *a*, Longitudinal section; *b*, cross-section.

layer-planes surrounded by an outer zone of layer-planes with circumferential orientation. Extensive oxidative etching in a plasma is said<sup>8</sup> to support this interpretation.

But no mechanism for the formation of the radial-circumferential structure has been proposed; and investigations using electron microscopy have provided positive evidence that layer-plane orientations in cross-sections of carbon fibres are random. Thus longitudinal sections cut from carbon fibres, made as described above and which in the optical microscope appeared to have a sheath and core, have always appeared uniform in the electron microscope; although selected area diffraction and (002) dark field images (Fig. 2*a*) showed that layer planes of the crystallites were parallel to the fibre axis, there was no preferred distribution between the two zones<sup>9</sup>. Moreover, tilting the specimen produced no overall change; radial-circumferential structures would have had pronounced effects. Transverse sections, ~50 nm thick, have recently confirmed that there was no preferred orientation of the layer planes in the fibre cross-section. Figure 2*b* shows that there were no obvious differences between the phase-contrast lattice-fringe images obtained from the core and sheath regions, and the continuous rings of the selected area diffraction pattern show that the orientations were random.

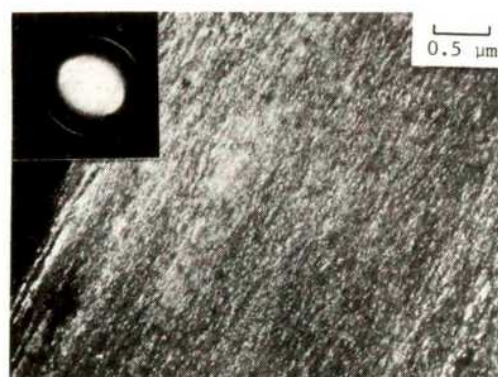
This raises the possibility that the optical anisotropy seen in the carbon fibres was caused not by structural anisotropy but by strain birefringence, and several recent observations support this.

First, optical activity and interference colours have been seen in reflected polarised light from the glassy carbon matrix in which carbon fibres had been embedded (see Fig. 3*a*); the glassy carbon was made from furfuryl alcohol resin carbonised to 1,000 °C only, a temperature at which stress graphitisation could not have occurred<sup>10</sup>. (Similar optical activity has been observed at the stressed interfaces formed when pieces of furfuryl alcohol resin-carbonised to 450 °C were coated with further resin and then carbonised at 1,000 °C.)

Second, cutting thin cross-sections of the carbon fibres caused knife damage; the shattered appearance (Fig. 3*b*) suggests differential residual stresses in the outer and inner zones.

Third, etching with argon ions for short erosion times, <60 min, caused similar amounts of erosion in the sheath and core and no features were seen to indicate any differences in structure even at magnifications of  $\times 20,000$ . Figure 4*a, b* shows both transverse and longitudinal sections, in which the lower erosion rate of the material between the sheath and core may have been due to its being unstressed.

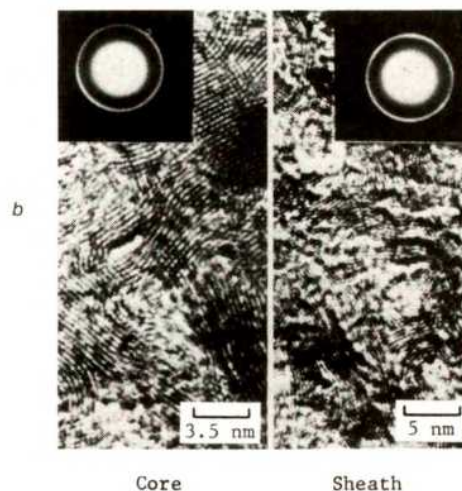
It is, therefore, suggested that the patterns in both the glassy carbon matrix and in the carbon fibres themselves are caused by strain birefringence. Furfuryl alcohol resin is thermosetting and shrinks by about 20% when heated to 1,000 °C; it forms a rigid cross-linked structure with no plastic flow<sup>11</sup> when it is carbonised. This shrinkage will cause a circumferential tensile stress in the matrix around a fibre. Also, it is thought that a radial oxygen gradient in incompletely oxidised PAN fibres may induce internal strains in the resulting carbon fibres. The carbon yield<sup>12</sup> from



a

Sheath Core

Interface assessed from optical micrographs



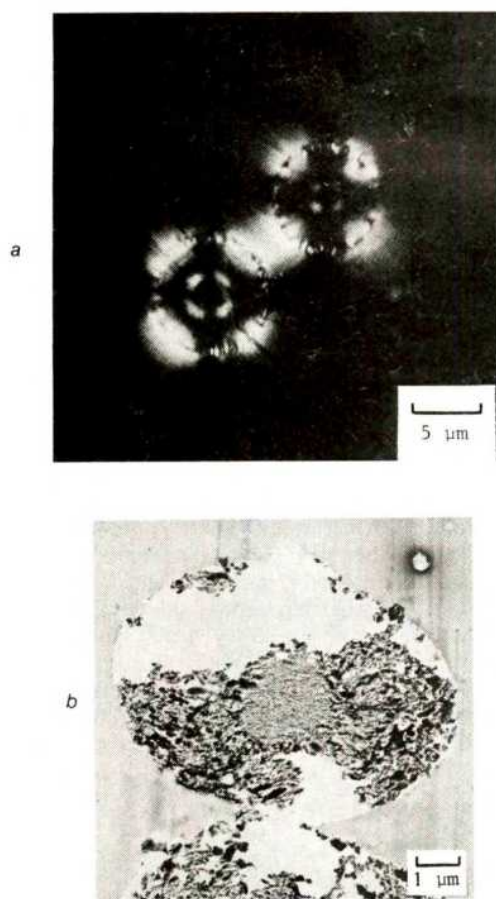
b

Core

Sheath

**Fig. 2** *a*, (002) Dark field electron micrograph of a thin longitudinal section. *b*, Electron diffraction and lattice-fringe images of a thin cross-section.





**Fig. 3** *a*, Polished cross-sections of carbon fibres in glassy carbon. Polariser and analyser crossed. *b*, A thin cross-section of a carbon fibre.

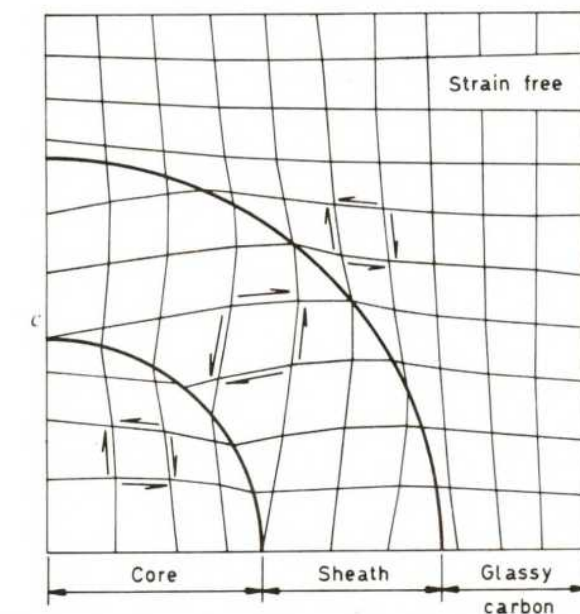
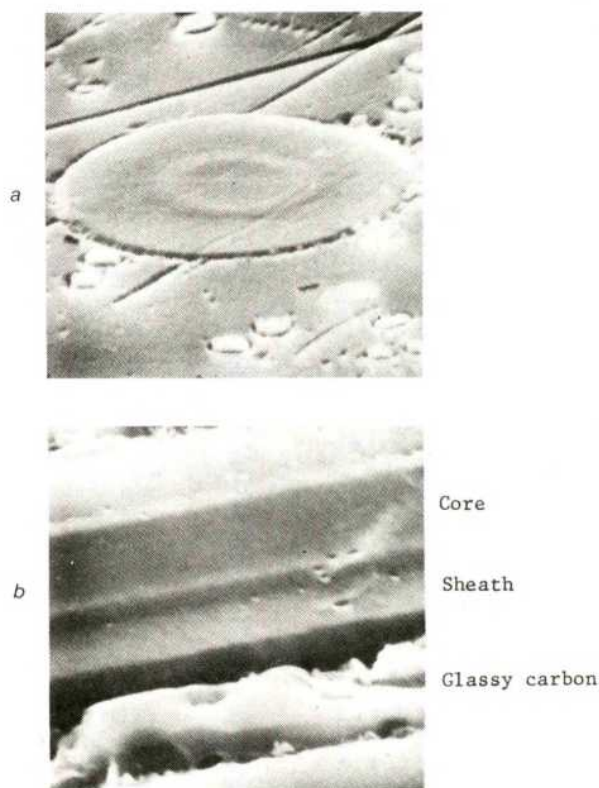
oxidised PAN fibres is about 50%, and that from unoxidised fibres about 30%; thus any differences in oxygen content will lead to density differences and probably also to differences in the transverse Young's modulus (the dependence of longitudinal Young's modulus on oxygen content is well established)<sup>4</sup>. When a partially oxidised PAN fibre is carbonised there will be a greater carbon yield in the oxygen-rich sheath than in the core. As the fibres do not melt or flow, as they are carbonised, the core will be restrained from shrinking by the more densely packed sheath so that a radial tensile stress will develop in the core with a corresponding circumferential compressive stress in the sheath. The region between the sheath and core is not stressed and seems optically isotropic.

The strain field of a carbon fibre embedded in a glassy carbon matrix (as in Fig. 3*a*) has cylindrical symmetry; hydrostatic stresses around and within each fibre lead to the shear strains shown in Fig. 4*c*.

It is suggested that the presence of a 'Maltese-Cross' pattern in the core of a fibre will depend on a non-uniformity of Young's modulus of the core carbon. If the Young's modulus were constant, the hydrostatic tensile stresses would deform the core evenly; each square of a grid in the core of Fig. 4*c* would be enlarged without distortion and the core would appear optically isotropic in the polarising microscope. But, if there had been an oxygen gradient then the transverse Young's modulus of the core carbon would decrease towards the centre of the fibre, the deformation of the core would be non-uniform and the maximum elongation would be circumferential giving the observed optical anisotropy, the pattern of the core having the same interference colours as that of the glassy carbon matrix; the distortion towards the centre of each fibre is too small to have an effect and it appears optically isotropic. This model also explains why the 'Maltese-Cross' pattern is less evident in micrographs of

carbon fibres made from well-oxidised PAN fibres as it is not seen when the oxidation kinetics do not lead to a significant oxygen gradient.

Transmission electron microscopy of thin transverse sections of carbon fibres made from PAN has shown that the structure is random; it is suggested that the observed optical anisotropy is due to stress in the fibres caused by differential shrinkage between the sheath and core. The optical anisotropy of carbon fibres made from different PAN precursor fibres often differs from a 'Maltese-Cross' and thin-sections of these are being examined.



**Fig. 4** Polished sections of carbon fibres in glassy carbon argon ion etched; *a*, cross-section fibre diameter 11.8 µm, etched 30 min; *b*, longitudinal section fibre diameter 10 µm, etched 60 min; *c*, a quadrant of the distortion in and around a carbon fibre embedded in a glassy carbon matrix.

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## Evidence for a widespread late Pleistocene humid period in the Kalahari

THE nature and extent of late Quaternary environmental changes in the interior of southern Africa are obscure, in part because of a lack of absolutely dated sediments, and landforms of clear palaeoenvironmental significance. Here I present evidence to show that, during the period 17,000–15,000 BP, the Kalahari, now a semi-arid area with little or no surface drainage, was a region of widely distributed small lakes, as a result of a substantial increase in rainfall.

A belt of pans (small dry or ephemeral lakes) with areas between 0.3 and 7 km<sup>2</sup> extends along the main Kalahari watershed, between latitudes 23–26°S (Fig. 1). The pans are situated

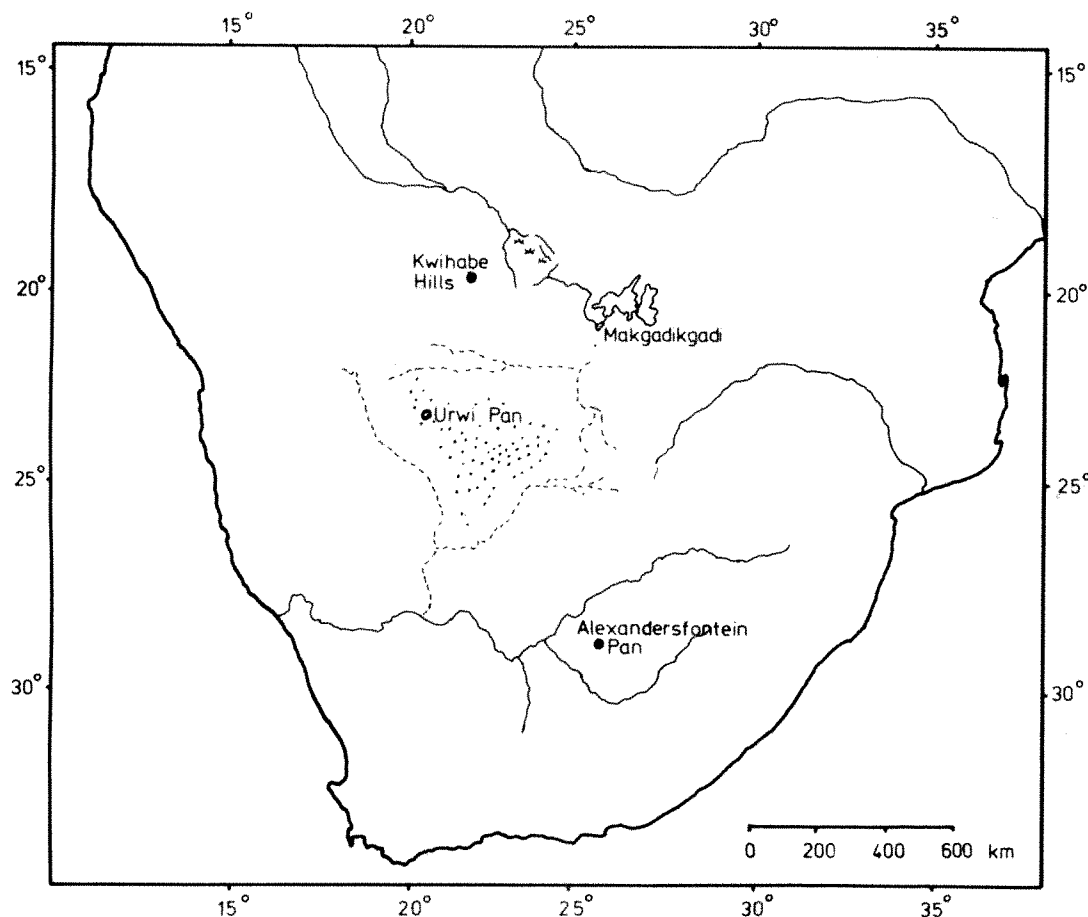
**Table 1** <sup>14</sup>C dates for Urwi pan stromatolites

Pta 2212	15,790 ± 110 BP
Pta 2213	15,610 ± 125 BP
Pta 2146	16,000 ± 160 BP
Pta 2150	16,255 ± 700 BP

in small, shallow, isolated enclosed depressions floored by calcareous and saline clays and sandy clays, laid down in shallow lacustrine conditions. Crescentic dunes on the southern margins of the pans indicate a deflation origin for the depressions<sup>1</sup>. The pans thus represent a microcosm of late Quaternary environmental change in the region, with deflation in arid or sub-arid conditions alternating with humid lacustrine conditions.

<sup>14</sup>C dates (Table 1) obtained from stromatolites recovered from a site 1–1.5 m above the present level of Urwi pan (Fig. 2) indicate that shallow lacustrine conditions prevailed in the pans 17,000–15,000 yr ago. The stromatolites are of spheroidal morphology, indicating their formation in permanent, wave agitated waters<sup>2</sup>. Changes in their internal structure suggest increased wave action during the period of their formation. The stratigraphic position of the stromatolites (Fig. 2) indicates that they pre-date the last phase of aeolian activity at the pans, and probably represent the final stages of full lacustrine conditions. Complementary sedimentary evidence<sup>1</sup> suggests that permanent alkaline lakes 2–3 times the present area of the pans were succeeded by increasingly arid conditions, in which saline clays were deposited in rapidly contracting seasonal or ephemeral lakes, on the margins of which a sandy wash was laid down. Subsequent deflation of the sandy deposits resulted in the formation of the inner dunes.

Today, mean annual rainfall in the area totals 300–400 mm, with pan evaporation exceeding 3,000 mm. It is thus not surprising that water bodies are ephemeral and runoff from the permeable surface sands negligible (<2 mm per yr). The existence of permanent lakes covering areas 2–3 times the area of



**Fig. 1** Southern Africa, showing location of pans, and sites mentioned in text.

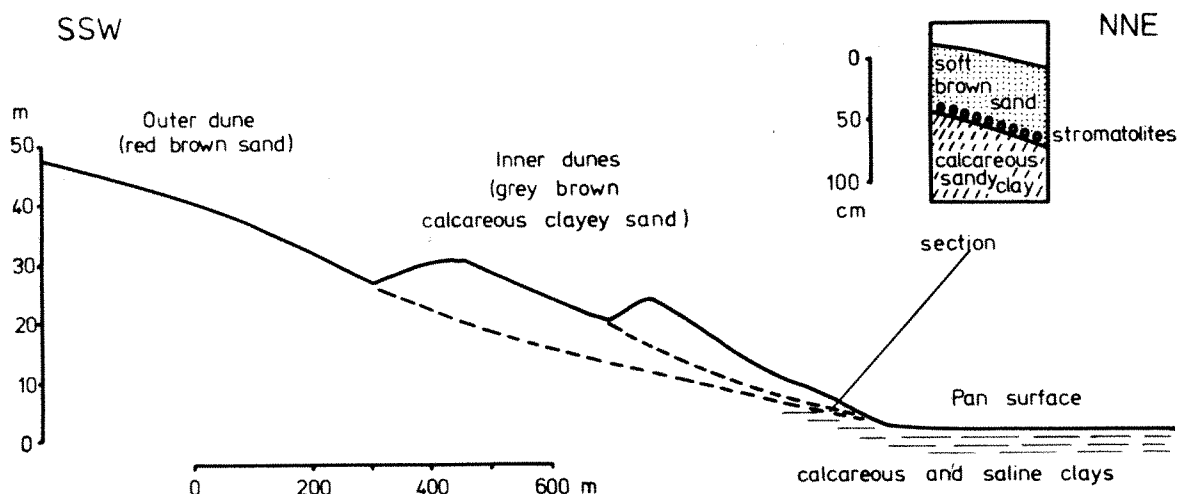


Fig. 2 Stratigraphic section at Urwi pan with location of stromatolites. All pans in region have an essentially similar stratigraphy.

the present pans is clear evidence that the Kalahari experienced substantially increased late Glacial rainfall, reduced evaporation, or a combination of both. Other workers in southern Africa<sup>3</sup> have assumed a 5–6 °C lowering of temperature at this time. By analogy with other stations in the interior of southern Africa with a mean annual temperature of 15–16 °C, compared to the 20–21 °C experienced in the southern Kalahari today, evaporation rates 17,000–15,000 yr ago would have been 40–50% lower. Similar comparisons, using data in Midgeley and Pitman<sup>4</sup> suggest that decreases in temperature and evaporation of this order would have led to increases in surface runoff to 10–20 mm per yr. Even allowing for this, rainfall increases of 1.5–2 times present amounts would have been necessary to sustain permanent lakes in the region, as the catchment areas for the lakes are extremely small (at most 10–15 km<sup>2</sup>). The potential contribution from shallow groundwater seepage is difficult to assess, but Verhagen *et al.*<sup>5</sup> demonstrate that significant recharge to shallow groundwater is taking place today in the northern Kalahari, where rainfall totals are 450–600 mm per yr. The increases in rainfall suggested must therefore be regarded as a maximum.

The evidence from the pans thus considerably extends the area of southern Africa known to have experienced increased late Glacial rainfall, and strongly suggests that the climate of the whole of the interior of the subcontinent was sub-humid at this time. Then the arid zone in southern Africa must have largely disappeared, except for isolated refuges along the Namib coast.

Butzer *et al.*<sup>3</sup> have calculated that rainfall totals were 123% of present amounts 16,000 yr ago in Alexandersfontein pan area near Kimberly (28°50' S); Cooke<sup>6</sup> estimated a threefold increase in rainfall for the Kwihe Hills area in northwestern Botswana (20° S), based on cave deposits with <sup>14</sup>C ages ranging from 14,000 to 17,000 BP. High lake levels in the Makgadikgadi depression some 20,000 years BP<sup>7</sup> may indicate that the period of increased rainfall started some 5,000 yr before this.

The picture of late Glacial climatic conditions in southern Africa which is now emerging is significantly different to that of the rest of the continent, which seems to have been dry at this time, with the southern margins of the Sahara up to 500 km south of their present position and low lake levels in east Africa; only parts of north Africa experienced wetter climates during this period.

The increased rainfall in the Kalahari at this time was probably the result, not of increased penetration of the subcontinent by winter cyclonic rains as Butzer *et al.*<sup>3</sup> suggest, but of greater summer rainfall due to a more southerly position of the Inter Tropical Convergence Zone. This was in turn the result of strengthened Northern Hemisphere circulations. Modern

parallels support this argument, with heavy rainfall in the region in recent years (1972, 1974–5, 1977–8) being associated with the persistence of the Inter Tropical Convergence Zone near its southerly limits (19–20° S). Desiccation of the interior of southern Africa was not well advanced until 9,000 BP, and was associated with much higher temperatures<sup>8</sup>, at a time when rainfall over much of Africa between the Equator and the northern Tropic was substantially greater than at present<sup>9</sup>. Equally interesting is the lack of correlation with Australia, where the late Glacial was cold, dry and windy<sup>9</sup>.

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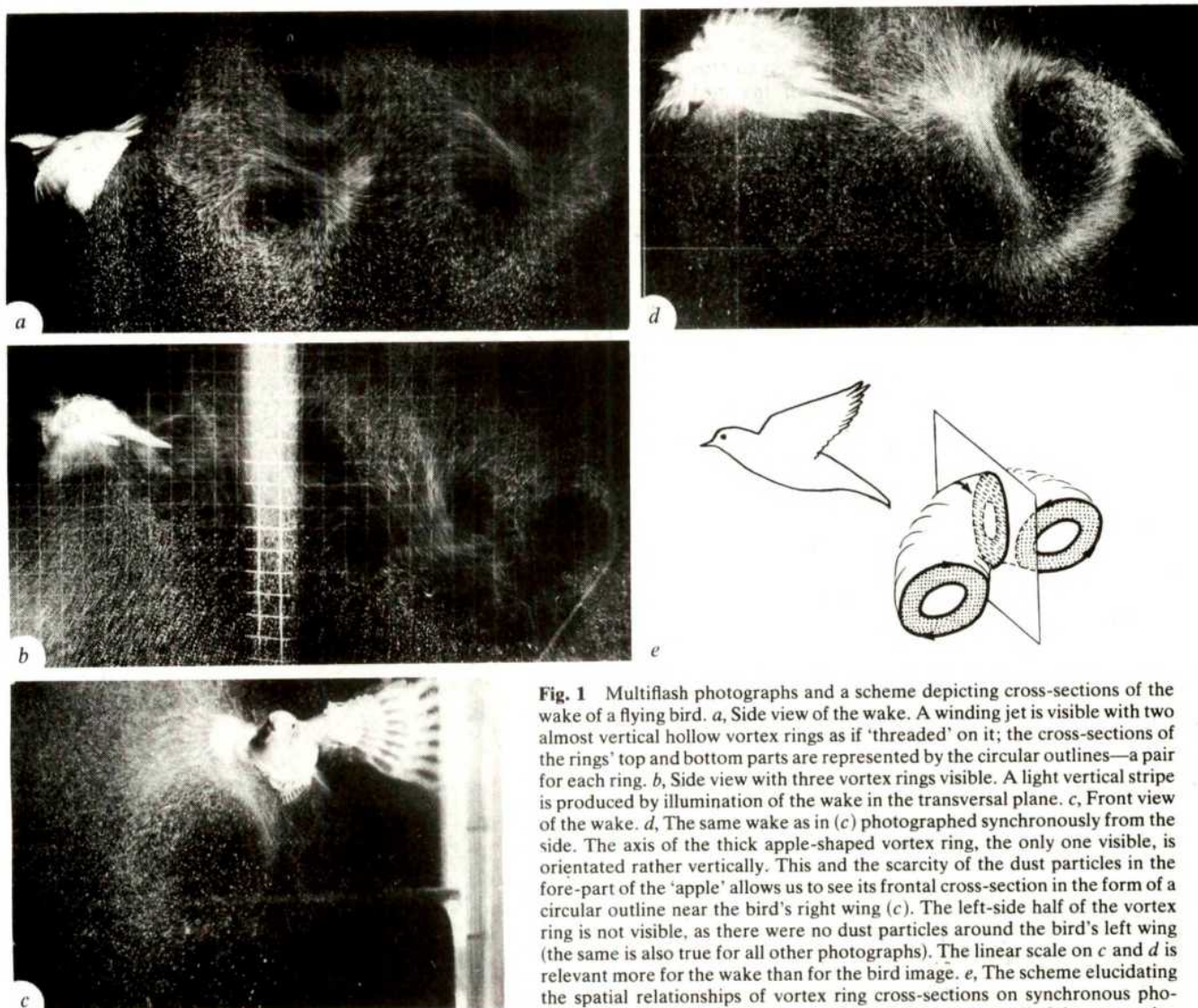
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## Tracing the wake of a flying bird

STUDY of the classical problem concerning “the way of an eagle in the air”, when applied to flapping bird flight, offers many difficulties. All dynamic interactions between an object and a fluid medium in which it moves are ciphered in the structure of its wake, but the wake behind a flying bird is difficult to work with because it is an invisible and very short-lived formation; moreover, its structure must be complicated as the bird's wings are working in a constantly changing manner. I report here the results of wake visualisation experiments carried out during short flights of a chaffinch (*Fringilla coelebs*) and a brambling (*F. montifringilla*) in enclosures. The general configuration of a wake is given, but no measurements have been made: in general, to extract some quantitative information from the wake structure, a more precisely documented picture of the dynamics of its formation is needed.





**Fig. 1** Multiflash photographs and a scheme depicting cross-sections of the wake of a flying bird. *a*, Side view of the wake. A winding jet is visible with two almost vertical hollow vortex rings as if 'threaded' on it; the cross-sections of the rings' top and bottom parts are represented by the circular outlines—a pair for each ring. *b*, Side view with three vortex rings visible. A light vertical stripe is produced by illumination of the wake in the transversal plane. *c*, Front view of the wake. *d*, The same wake as in (*c*) photographed synchronously from the side. The axis of the thick apple-shaped vortex ring, the only one visible, is orientated rather vertically. This and the scarcity of the dust particles in the fore-part of the 'apple' allows us to see its frontal cross-section in the form of a circular outline near the bird's right wing (*c*). The left-side half of the vortex ring is not visible, as there were no dust particles around the bird's left wing (the same is also true for all other photographs). The linear scale on *c* and *d* is relevant more for the wake than for the bird image. *e*, The scheme elucidating the spatial relationships of vortex ring cross-sections on synchronous photographs (*c, d*); as in the photographs only the right-side half of the vortex ring is shown. The cross-sections in the plane of the drawing correspond to the side view of the wake (*d*) and the one at right angles to this plane represents the front view of the wake (*c*). The direction of the vortex ring circulation is indicated by the arrows. The pictures were taken in a net cage (*a, b*) and in a large aquarium (*c, d*); the birds photographed were brambling (*b*) and chaffinch (*a, c, d*); the number of flashes per series was about 13 (*a*), 8 (*b*) and 7 (*c, d*); flash frequencies were 1 kHz (*a, b*) and about 0.75 kHz (*c, d*); visualisation was carried out with paper (*a, b*) and wood (*c, d*) dust.

The method involved multiple flash photography of moving small light particles forming a cloud through which the bird was forced to fly. Several visualising agents were tried but wood and paper dusts manufactured on a grindstone were most successful. Mean diameters of individual particles of both types of dust were 0.5–0.6 mm (paper dust had a more pronounced tendency to aggregate in flakes of a mean diameter of  $\sim 1.0$  mm), but average sinking velocities were different:  $0.5 \text{ m s}^{-1}$  in wood and  $0.2 \text{ m s}^{-1}$  in paper dust. Birds performed their short flights between two perches in a Plexiglass aquarium ( $2 \times 0.5 \times 1 \text{ m}$ ) and in a net cage ( $2.6 \times 0.6 \times 0.7 \text{ m}$ ). An electronic generator producing a series of flashes was designed for the study. The generator was used in a waiting regime and was triggered from one or two photorelays as the bird crossed an infrared beam. The frequency of flashes and their number per series were widely adjustable. Satisfactory results were obtained with frequencies of the order of 750–1,000 Hz and 6–8 flashes per series.

The experimental procedure was as follows. The room was darkened (only very faint illumination being retained) and the shutters of two photographic cameras were opened. Then a cloud of flow-visualisation particles was blown out with the aid of a long rubber tube from a container attached above the middle part of a bird's trajectory. At the same time the bird was flushed from the perch. The flying bird triggered the flash

generator and hence a wake photograph was produced. Cameras were then prepared for the next exposure and the cycle repeated.

In the photographs obtained the multiple images of individual particles clearly show directions and relative velocities of their movement in the plane of the paper. The faster a particle is moving the longer the segments of a broken line it produces. One can consider these lines as an approximation to the real trajectories of fluid particles. With the linear scale and flash frequencies known it is possible to calculate roughly a number of characteristic velocities in the plane of the wake flow field pictured.

To analyse the photographs obtained (Fig. 1) it is necessary to consider some peculiarities of the methods used. Photographic superposition of successive images causes a strong impression as if light objects, specifically dust particles, are always situated in front of dark ones. The bird itself moves relatively slowly, so its image is usually overexposed with few details visible—exposures being chosen to reproduce primarily the wake structure. Under the action of a centrifugal force the core parts of rotating bodies of air are free from visualising material. This causes accentuation of such structures, but it should be remembered that their apparent dimensions are also strongly dependent on the size and density of visualising particles. Finally, the

particle cloud was formed by the experimenter in such a way that it embraced only the wing of the bird remote from the observer, so that sections of the wake were produced on the photographs.

The two-dimensional longitudinal view of the bird wake conforms reasonably well to the reversed vortex street with a 'reactive' jet between the vortices—the pattern that was predicted long ago by theorists and then repeatedly reproduced in experiments with an oscillating aerofoil<sup>1</sup>. Analysis of the photographs also reveals that the spatial structure of the wake of an actively flying bird is represented by a series of thick and more or less deformed vortex rings with an intense and winding jet which passes through the rings' holes. Form and orientation (that is, axis inclination) of the vortex rings are variable because the photographs correspond to different stages of birds' short flight with different requirements on lift and thrust and unequal contribution by the ballistic component (the initial jump from the perch) at each stage.

The development of a vortex ring can be conceived in the following way. The upper part of the ring (if it is placed vertically) is formed by the starting vortices of both the wings at the beginning of their downstroke; these are extended on the sides of the ring, during the middle stages of the downstroke, by the wings' tip vortices. The lower part of the ring is formed by the stopping vortices shed from the wings in their lowest position at the bottom of the downstroke. The vortices generated by the left and right wing are closed against each other at the upper and lower points of the wing stroke. So a vortex ring is formed. In most if not all passerine birds all the aerodynamic work is known to be done only on the downstroke; on the upstroke the wings are folded and their interaction with the air is minimal<sup>2</sup>. This is why vortex rings in the wake of the finches studied here are produced only during the downstroke.

A recent theoretical model<sup>3,4</sup> has predicted the configuration of the vortex rings observed in these experiments as being the most realistic description of the wake of a flying bird, consistent with the unsteady regime of its wing beat. This model provides a framework for calculations of the power consumed in flight and for the quantification of the vortex distribution of the wake.

I thank Professor Sir James Lighthill for the kind attention he has given to this report and V. I. Petrowsky for designing the generator and helping to produce the photographs.

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## Shoot height, weight and standing crop in relation to density of monospecific plant stands

DESPITE great differences in architecture and supporting tissue, monospecific stands of single-stemmed plants ranging in size from a moss to a tree follow a simple rule relating shoot dry weight and standing crop to density. We describe here an equation for this relationship which remains true for almost any fully occupied plant stand dominated by a single species.

Data were obtained, mostly from the literature<sup>1–19</sup>, for 65 stands of 29 different plant species (Table 1) ranging from mature midsummer shoots of annual and perennial non-woody species to saplings and full-sized trees. Figure 1a shows the

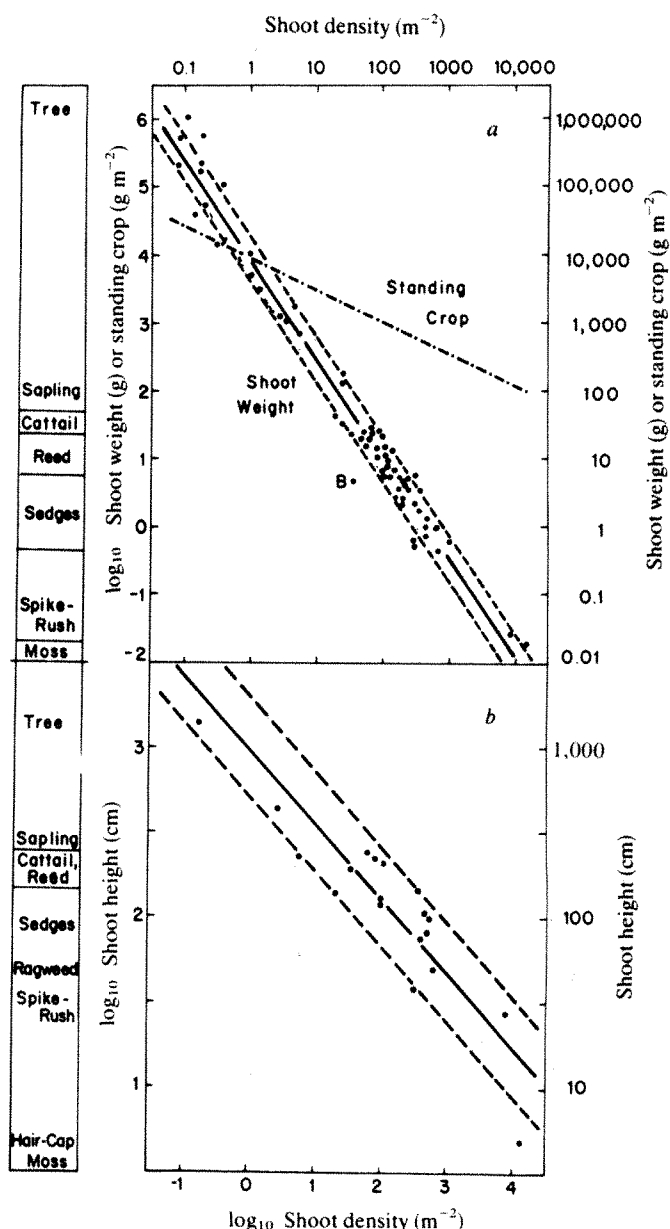


Fig. 1 The relationship of shoot dry weight and standing crop (a) and height (b) to density in monospecific stands of plants. Dashed boundary lines at 0.3  $\log_{10}$  intervals below and above the solid lines represent plants half and twice as large. Point B represents *Bolboschoenus maritimus*.

inverse, double logarithmic relationship of shoot dry weight ( $w$ , grammes) to density ( $d$ ,  $m^{-2}$ ). It is calculated as the reduced major axis<sup>20,21</sup>, because neither variable is truly dependent on the other, and has the form  $w = 9,670d^{-1.49}$  and the correlation coefficient  $r_{\log 10} = -0.987$ .

A simple geometric model, developed by Yoda *et al.*<sup>1</sup>, to explain intraspecific weight/density relationships during self-thinning of overcrowded stands of individual plant species, seems also to apply to interspecific weight/density relationships among diverse species, ranging in shoot weight over more than seven orders of magnitude and in shoot density over five orders of magnitude. The model is described as follows: (1) assume that available space is fully occupied (that is, that the leaf canopy is closed), and that the area occupied by a plant is proportional to the square of some linear dimension (for example, basal diameter or height); (2) then weight (or volume) per shoot will be proportional to the cube of the linear dimension; (3) weight per shoot is therefore proportional to the 3/2 power of the area occupied or to the -3/2 power of its reciprocal, which is density.



This exponent of  $-1.5$  is very close to that observed in Fig. 1a ( $-1.49$ ), which therefore appears to fit the suggested model despite major differences in plant architecture (for example, *Polytrichum*, *Pteridium*, *Carex*, *Typha*, *Phragmites*, *Ambrosia*, *Betula*, *Abies*), and in the nature of supporting tissues, with a large component of parenchyma in some species (for example, *Eleocharis*, vegetative shoots of *Typha*) and of sclerenchyma in others (*Abies*, *Betula*).

The relationship may be followed further by examining the ranges of shoot height and weight (Table 2). The rules of the simple geometric model dictate that the range for  $\log_{10}$  height ought theoretically to be half the range for  $\log_{10}$  density, whereas the range for  $\log_{10}$  weight ought to be 1.5 times the range for  $\log_{10}$  density. The observed ranges are in good agreement with the model, being within 7% of the theoretical range for height, and within 2% of the theoretical range for weight. Conformity would probably be greater if height data were available for the largest *Abies sachalinensis*. It is 6.5 times the weight of *Taxodium distichum*, the largest plant for which height data are available. According to the model, 6.5 times greater weight implies 1.87 times greater height, and an increase of 0.27 in the  $\log_{10}$  height range. Adding 0.27 to 2.44 gives 2.71, within 4% of the theoretical height range for all 27 species.

The relationship of shoot height ( $h$ , cm) to density ( $d$ ) of 15 species in 19 stands is shown in Fig. 1b, and is given by the reduced major axis,  $h = 970d^{-0.443}$ . The correlation coefficient,  $r_{\log 10} = -0.902$ . Thus, density explains only 81% of the variance in shoot height, as compared with 98% of the variance in weight for the same 19 stands. Marked architectural differences are reflected in the ratios of height to cube-root of dry weight among the different species. Ratios for seven species of *Carex* range from 67 to 130, whereas two species of *Typha* have ratios of 40 and 68. Three stands of *Phragmites communis* range from 76 to 93. Trees exhibit lower ratios, 39 and 26 for *Pinus silvestris* and 26 for *Taxodium distichum*. The moss *Polytrichum commune* has the lowest ratio, at 19.

Table 1 Species investigated\*

<i>Abies sachalinensis</i> <sup>1</sup>	<i>Glyceria maxima</i> <sup>2</sup>
† <i>Ambrosia artemisiifolia</i> (unpubl.)	† <i>Phragmites communis</i> <sup>2,9-13</sup>
<i>Ammophila arenaria</i> (unpubl.)	† <i>Pinus silvestris</i> <sup>14</sup>
<i>Betula</i> ( <i>platyphylla</i> , <i>Ermani</i> , <i>Maximowicziana</i> ) mixed <sup>1</sup>	† <i>Polytrichum commune</i> (unpubl.)
<i>Bolboschoenus maritimus</i> <sup>2</sup>	<i>Pteridium aquilinum</i> <sup>15</sup>
† <i>Carex acuta</i> <sup>3</sup>	<i>Scirpus fluviatilis</i> <sup>8</sup>
<i>C. atherodes</i> <sup>4</sup>	<i>S. validus</i> <sup>8</sup>
† <i>C. elata</i> <sup>3</sup>	† <i>Sparganium erectum</i> <sup>16</sup>
† <i>C. lacustris</i> <sup>3,4,5</sup>	<i>S. eurycarpum</i> <sup>8</sup>
<i>C. lanuginosa</i> <sup>4</sup>	† <i>Taxodium distichum</i> <sup>17</sup>
† <i>C. lasiocarpa</i> <sup>3,4</sup>	<i>Typha angustifolia</i> <sup>2,13</sup>
† <i>C. rostrata</i> <sup>3,4,6,7,8</sup>	<i>T. glauca</i> <sup>8</sup>
† <i>C. vesicaria</i> <sup>3</sup>	† <i>T. latifolia</i> <sup>18,19</sup>
† <i>Eleocharis calva</i> (unpubl.)	† <i>T. (angustifolia, glauca, latifolia)</i> mixed, (unpubl.)

\*Other species accounted only for a few % of above-ground standing crop and less than 10% in all measured cases.

†Data for both weight and height were available.

The weight/density relationship within a monospecific plant stand undergoing competitive self-thinning is generally characterised<sup>1</sup> by a negative exponent of  $-1.5$ . Different species are expected<sup>1,22</sup> to exhibit distinct differences in the constant  $C$  of the equation  $w = Cd^{-1.5}$ . Several species should thus yield a family of curves, all with negative exponents near  $-1.5$ , but with different values for the constant  $C$  presumably reflecting differences in plant architecture. However, within the limits of variability normally observed for such an inverse power-law relationship, most species fit reasonably well to the solid line in Fig. 1a, defined by a single value (9,670) of the constant  $C$ . Three-quarters of the 65 plant stands in Fig. 1a lie within the

dashed boundary lines shown 0.3  $\log_{10}$  intervals below and above the fitted solid line. These boundary lines ( $C = 4,900$  and 19,500 respectively) represent shoots one-half and twice as large as those which fit the equation given earlier. Moreover, data for many individual species reveal a variability at least as great as that represented by the dashed boundary lines. Data from laboratory experiments may be similarly variable. Two populations of *Helianthus annuus* allowed to self-thin at full light intensity<sup>22</sup> exhibited  $C$  values of 6,920 and 91,200 respectively, and regression exponents of  $-1.33$  and  $-1.84$ . The clumped distribution of many plant shoots and the often logarithmic frequency distribution of shoot sizes in overcrowded stands<sup>1</sup> contribute greatly to the substantial variability.

Table 2 The ranges of height\*, density† and weight† for the plant shoots in Fig. 1

	Minimum	Maximum	$\log_{10}$ range	
			Actual	Theoretical
Height (cm)	5.0	$1.39 \times 10^3$	2.44	2.62
Density ( $m^{-2}$ )	0.079	$1.38 \times 10^4$	5.24	—
Dry weight (g)	0.019	$1.07 \times 10^6$	7.75	7.86

\*19 stands, 15 species. †65 stands, 27 species.

Architecturally diverse species overlap considerably, and where deviations from the reduced major axis occur they do not seem to be related in any consistent way to differences in plant architecture or in the nature of the supporting tissues. A number of self-thinning curves in the literature<sup>1,22</sup> also conform rather well to the relationship shown in Fig. 1a, especially if reasonable corrections are made for below-ground material of whole plants. Even if more detailed study reveals systematic variations in the constant  $C$  which can be related to differences in plant architecture, Fig. 1a suggests that its values will lie within a fairly narrow range, and mostly between half and twice the value reported here.

Two species, *Chenopodium album*<sup>1</sup> and *Bolboschoenus nigricans* (B in Fig. 1a), appear to deviate substantially from the relationship reported here. The deviation of *Chenopodium* is particularly striking because of the conformity of a similar weed, *Amaranthus retroflexus*<sup>1</sup>. However, according to one of the authors (T. Kira), their *Chenopodium* data were for fresh weight, others were for dry weight. *Bolboschoenus* (= *Scirpus*) *maritimus*, marked B in Fig. 1a, lies well below the reduced major axis. This rhizomatous species colonises open water, and probably had not developed a closed canopy. The relationship will also break down where aridity and cold prevent development of a complete plant cover, as in deserts and tundra.

Self-thinning is not always characteristic of monospecific stands of plants in nature, particularly in uncrowded situations. The exponent in the weight/density relationship is generally close to  $-1.0$  in such cases, so that standing crop per unit area is independent of density. This is the 'Law of Constant Final Yield'<sup>23</sup> and this holds for stands of *Taxodium distichum* in Georgia<sup>17</sup> and *Phragmites communis* in Czechoslovakia<sup>12</sup>. The reduced major axes for both species lie close, although at an angle, to their respective short segments of the fitted line in Fig. 1a. Apparently this line sets an approximate upper limit to the mean size of plant shoots at any given density, whether or not self-thinning is taking place.

The negative slope in Fig. 1a obviously reflects structural constraints, with a less dense plant spacing allowing a larger basal area for stem support, and hence a greater ultimate height and mass. This structural explanation might be thought to have an underlying basis in the physiological balance between respiration, a function of shoot weight, and photosynthesis, a function of leaf area. In such a case, shoot weight ought to be



closely related to leaf area index. However, leaf area indices calculated from data compiled by R. H. Whittaker<sup>24</sup> show little difference in communities with very different shoot densities and sizes. For example, temperate and tropical evergreen and deciduous forests exhibit mean indices between 5 and 12 m<sup>2</sup> m<sup>-2</sup>, as compared to indices of 3.6 for temperate grasslands and 7.0 for swamps and marshes. Grassland and wetland communities have far smaller shoot weights and greater shoot densities than forests. Moreover, fourteen Czechoslovak stands of *Phragmites communis*<sup>12</sup> exhibit no relationship of leaf area index, which ranges from 2.4 to 6.3, to either shoot weight or density, which correlate strongly with one another ( $r_{\log 10} = -0.91$ ). White<sup>25</sup> discusses the point in more detail.

It follows from the weight/density relationship in Fig. 1a that large plants growing at low density yield a larger above-ground standing crop than small plants at high density, with  $wd = 9,670d^{-0.49}$ . This relationship is shown as a dash-dot line in Fig. 1a. It can be seen that standing crop at a density of 0.1 shoots m<sup>-2</sup> (characteristic of a large tree) is 26,000 g m<sup>-2</sup>, whereas at a density of 10,000 shoots m<sup>-2</sup> (characteristic of a large moss), it is 120 g m<sup>-2</sup>. Only widely spaced plants, which can develop a large basal area and so grow to great heights, are able to accumulate large amounts of biomass. This is so obvious as to be a truism. The remarkable fact is that a single, simple rule relating shoot weight and standing crop to density should apply over so many orders of magnitude and to plants as diverse in their architecture as mosses, ferns, gymnosperms, monocotyledons and dicotyledons.

After submitting this article I learned from J. L. Harper that a similar relationship was described at the Twelfth International Botanical Congress in Leningrad by J. White, who subsequently informed me that it will be dealt with further in a forthcoming publication.

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## Parasite pathogenicity and the depression of host population equilibria

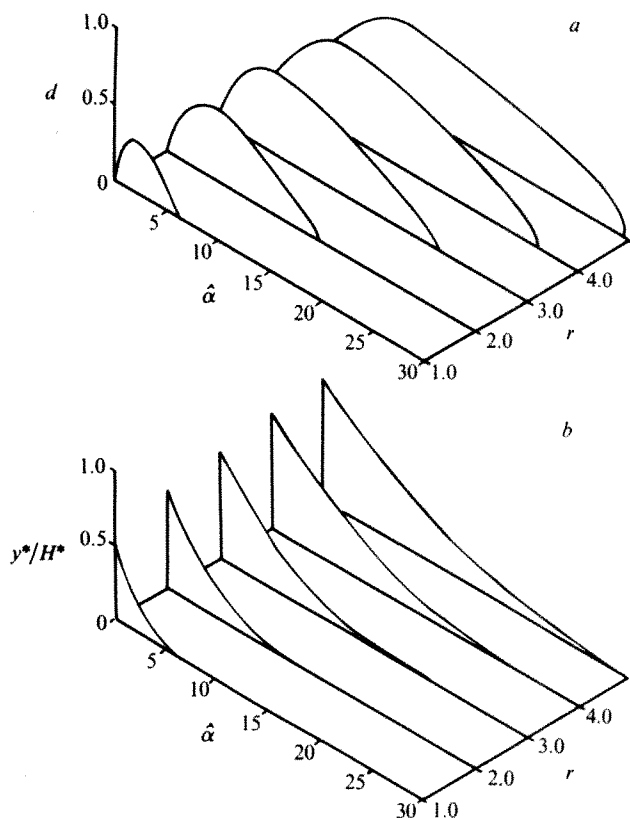
THE use of pathogens as agents for the biological control of pest species has increased rapidly in recent years with the realisation that chemical pesticides are not the panacea they were once thought to be<sup>1-6</sup>. The selection of a suitable parasite is often based on the assumption that highly pathogenic organisms will be most effective in minimising pest population growth<sup>6-8</sup>. Recent theoretical studies of the dynamics of host-parasite associations provide a template for testing this assumption<sup>9-12</sup>. This paper examines the relationship between parasite pathogenicity, as measured by the severity of the pathogen's influence on host survival, and the consequent depression of the host population from the size it would have achieved in the absence of infection. The maximum degree of depression is achieved by direct life cycle parasites of moderate to low pathogenicity, a prediction which has important implications for the use of pathogens as biological control agents. In agreement with traditional beliefs<sup>13,14</sup>, theory suggests that highly pathogenic species will cause their own extinction but not that of their host. The relationship between population depression and host reproductive potential is shown to be critically dependent on the biological characteristics of the pathogen.

Parasites can be divided into two main classes. Microparasites (viruses, bacteria, protozoans) are characterised by small size, short generation times, extremely high rates of direct reproduction within the host, and a tendency to induce lasting immunity to re-infection in those hosts that survive initial infection<sup>15</sup>. The duration of infection is typically short in relation to the expected lifespan of the host, and thus is of a transient nature (there are exceptions to this general trend, as shown, for example, by herpes simplex virus<sup>15</sup> and the slow viruses<sup>16</sup>). Macroparasites (parasitic helminths and arthropods) have much longer generation times, and direct multiplication within or on the host is either absent or occurs at a low rate compared with that of microparasites.

Such immune responses as are elicited by these metazoans tend to depend on the number of parasites present in a given host and to be of relatively short duration relative to the expected lifespan of the host<sup>17-19</sup>. Macroparasitic infections therefore tend to be of a lasting nature, as the hosts are susceptible to continued re-infection.

Both microparasites and macroparasites may complete their life cycles by passing from one host to the next either directly or indirectly via one or more intermediate host species. Pathogens which have been used in biological control<sup>1-3</sup>, and those whose potential is now being investigated<sup>4-8</sup>, all exhibit direct life cycles (various viruses, bacteria, protozoa and nematodes).

The influence of parasite pathogenicity (*per capita* rate of parasite-induced host mortality) on the dynamics of host population growth may be explored by means of population models framed in differential equations. The mathematical theory of the dynamics of microparasitic infectious diseases, as recently reviewed by Bailey<sup>20</sup>, is largely based on compartmental models which delineate susceptible, infected and immune categories of hosts (containing, respectively,  $x$ ,  $y$  and  $z$  numbers of hosts). The model used to examine the dynamics of microparasitic infections differs from standard epidemiological models in assuming that the total number of hosts,  $H = x + y + z$ , is not treated as some independently set constant, but is assumed to be a variable determined by both the dynamics of the disease and the natural *per capita* birth rate,  $a$ , and death rate,  $b$ , of the host. It is also assumed that in the absence of infection the disease-free population grows logistically<sup>21</sup> to an environmental carrying capacity,  $K$ , where  $K = (a - b)/\beta$  (the constant  $\beta$  measures the severity of density-dependent constraints on population growth). The system of equations for susceptible  $x$ , infected  $y$  and immune  $z$



**Fig. 1** Microparasitic infection model. Following conventional lines, the rate at which hosts acquire infection is assumed to be proportional to the number of encounters between susceptible and infected hosts, being  $\Lambda xy$ , where  $\Lambda$  is a transmission coefficient<sup>20</sup>. The mortality rate of infected hosts is taken to be  $b + \hat{\alpha}$ , with  $\hat{\alpha}$  representing the mortality caused by the disease; there is also a recovery rate from infection,  $v$ . Recovered hosts are assumed to be initially immune, but this immunity can be lost at a rate  $\gamma$  (for permanent immunity  $\gamma = 0$ ). These assumptions lead to the following equations:  $dx/dt = aH - (b + \beta H)x - \Lambda xy + \gamma z$ ,  $dy/dt = \Lambda xy - (b + \beta H)y - \hat{\alpha}y - v y$ ,  $dz/dt = v y - (b + \beta H)z - \gamma z$ . The construction of these equations follows conventional lines<sup>20</sup> (with the parameters  $a$ ,  $b$  and  $\beta$  as defined in the main text) except for the inclusion of a *per capita* density-dependent host mortality rate  $(b + \beta H)$ <sup>21</sup>. The sum of the three equations for  $x$ ,  $y$  and  $z$  yields:  $dH/dt = (a - b - \beta H)H - \hat{\alpha}y$ , equation (1). Provided  $(a - b)$  is positive, the model yields locally stable equilibria. If host density,  $H$ , is less than a threshold value,  $H_T$ , where  $H_T = (b + \hat{\alpha} + v)/(\Lambda - \beta)$ , then after the introduction of infected hosts into the population,  $y$  will initially decrease and  $x$  increase. However, once  $x$  exceeds  $H_T$ , then  $y$  will increase and the system will converge (steadily or with damped oscillations) on one of two types of stable equilibrium state. Stable co-existence of host and parasite, where  $H^* < K$  and  $y^* > 0$ , will result provided  $\Lambda > \beta[1 + (b + \hat{\alpha} + v)/(a - b)]$ . If this condition is not satisfied the parasite fails to co-exist and  $H^* = K$  and  $y^* = 0$ . *a*, The influence of parasite pathogenicity,  $\hat{\alpha}$ , and the natural intrinsic growth rate of the host population  $r$ , where  $r = a - b$ , on the degree of host population depression  $d$ , where  $d = 1 - H^*/K$ . Parameter values of the model are as follows:  $\beta = 0.01$ ,  $\Lambda = 0.1$ ,  $\gamma = 0.05$ ,  $v = 0.5$ . *b*, The influence of  $\hat{\alpha}$  and  $r$  on the equilibrium proportion of infected hosts,  $y^*/H^*$ . Parameter values as for *a*.

hosts and their dynamical properties are detailed in the legend to Fig. 1. The model has two distinct patterns of population behaviour, namely (1) the disease persists and regulates the host population at a locally stable equilibrium  $H^*$ , where  $H^* < K$ , and  $y^* > 0$ ; (2) the infection is not maintained within the host population and a stable disease-free equilibrium results where  $H^* = K$  and  $y^* = 0$ .

The relationship between parasite pathogenicity, measured by  $\hat{\alpha}$ , the *per capita* rate of disease-induced mortality in the infected class of hosts, and the degree of depression of the host population equilibrium,  $d$ , where  $d = 1 - H^*/K$  (varying

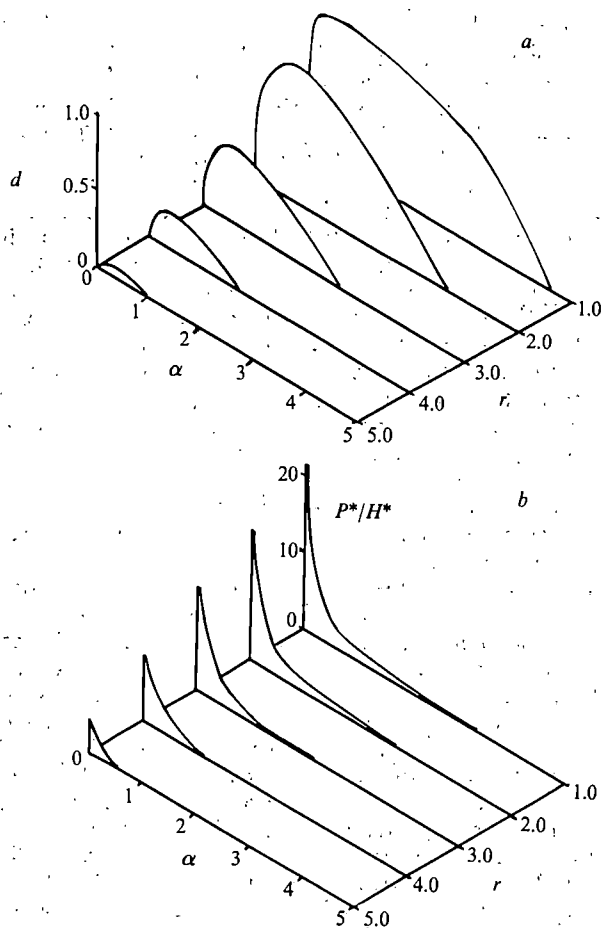
between 0 and 1), is shown in Fig. 1*a* for various values of  $r$ , the natural intrinsic growth rate of the host population ( $r = a - b$ ). For a fixed value of  $r$ , the maximum degree of depression is achieved by parasites of moderate to low pathogenicity. Increased rates of parasite-induced host mortality result in a greater degree of host population depression until the rate of loss of infected hosts begins to have a detrimental effect on the efficiency of disease transmission. When the level of pathogenicity is very high, infected hosts die before effective transmission is achieved and the disease is thus unable to persist within the host population ( $y^* \rightarrow 0$ ,  $H^* \rightarrow K$ ). In brief, highly pathogenic organisms are likely to cause their own extinction but not that of their host. These conclusions withstand a variety of changes in model structure. Most importantly, if a totally immune category of hosts is absent, recovered hosts passing directly back to the susceptible class (as may often be the case with microparasitic infections of arthropods), a pattern emerges which is similar to that shown in Fig. 1.

Macroparasites with direct life cycles tend to produce persistent infections where the pathogenicity to the host, the rate of production of transmission stages and any resistance of the host to further infection all typically depend on the number of parasites present in a given host<sup>11,17-19,22</sup>. A crude division of the host population into susceptible infected and immune classes is therefore inappropriate and a description of the dynamics must deal with the full probability distribution of parasites within the host population. Anderson and May<sup>9</sup> have studied the dynamics of macroparasitic diseases with the aid of three differential equations for the number of hosts  $H$ , parasites  $P$ , and free-living infective stages  $W$ , which incorporate certain statistical moments of the distribution of parasite numbers per host. Parasite pathogenicity is measured by a parameter,  $\alpha$ , which defines the disease induced *per capita* host death rate, per parasite, as a large amount of empirical evidence suggests that the rate of host mortality is proportional to parasite burden in a given host<sup>9,11,22</sup>.

A model based on the work of Anderson and May<sup>9</sup> but incorporating the assumption that in the absence of infection ( $P = 0$ ) the host population grows in a logistic manner as defined in equation (1) (the details of the model are given in the legend to Fig. 2), predicts a similar relationship between parasite pathogenicity,  $\alpha$ , and host population depression,  $d$  (Fig. 2), to that derived for microparasitic infections (Fig. 1). A further similarity in the predictions of the two models is shown in Figs 1*b* and 2*b*. Increased pathogenicity results in a decrease in both the proportion of infected hosts ( $y^*/H^*$ , microparasitic infections) and the mean parasite burden ( $P^*/H^*$ , macroparasitic infections). Both models suggest that observed levels of prevalence and intensity of infection within host populations in natural habitats will closely reflect parasite pathogenicity, a prediction supported by empirical evidence<sup>23</sup>.

In contrast to these similarities, the impact of high rates of host population growth in the macroparasitic infection model is to lessen the degree of depression relative to the disease-free equilibrium value  $K$ , whereas the exact converse is predicted by the microparasite model. The explanation of this paradox lies in the ability of microparasites to colonise a host by direct multiplication within the host. The rate of establishment of new colonies in uninfected hosts is therefore enhanced by high host birth rates which continually replenish the stock of susceptible individuals. High rates of establishment increase the proportion of infected hosts in the population and thus increase the degree of population depression. Experimental studies of viral and bacterial infections in mouse populations clearly illustrate this principle<sup>24</sup>.

In contrast, the population growth of macroparasites, which do not tend to exhibit direct multiplication, is dependent on the gradual accumulation of new infections within individual hosts and not on the input of susceptible individuals to the host population. High host birth rates tend to offset the mortalities caused by infections and hence decrease the degree of host population depression. Substantial depression of host popu-



**Fig. 2** Macroparasitic infection model. The framework of the macroparasitic model is based on the work of Anderson and May<sup>9,11</sup>, where the three equations for the population of hosts  $H$ , parasites  $P$ , and infective stages  $W$  are as follows:  $dH/dt = (a - b - \beta H)H - \alpha P$ ,  $dP/dt = \Lambda WH - (\mu + b + \alpha + \beta H)P - (\mu + \alpha)(k + 1)P^2/(kH)$ ,  $dW/dt = \lambda P - cW - \Lambda WH$ . The host birth and death rates  $a$  and  $b$  are as defined earlier for the microparasite model, as is the transmission parameter  $\Lambda$  (hosts acquire individual parasites at a rate proportional to the number of contacts between hosts and parasite infective stages,  $\Lambda WH$ ) and the density-dependent host mortality rate,  $b + \beta H$ . The parasite-induced host death rate is assumed to be linearly proportional to the parasite burden in a given host and to occur at a rate  $\alpha$  per parasite. The parasites are distributed as a negative binomial distribution with parameter  $k$ ;  $\mu$  is the natural mortality rate of adult parasites, the net rate being dependent on the density of parasites within an individual host;  $\lambda$  is the rate of production of infective stages by an adult parasite, and  $c$  is the death rate of these infective stages. The empirical basis of these equations has been described in detail elsewhere<sup>9-11</sup>. Provided  $(a - b)$  is positive, the model yields locally stable equilibria. If host density  $H$  is less than a threshold value  $H_T$ , where  $H_T = c(\mu + a + \alpha)/[\Lambda(\lambda - \mu - a - \alpha)]$ , then the parasite cannot become established ( $dP/dt < 0$ ). However, if  $H > H_T$ , provided  $\lambda - (a + \mu + \alpha) > c\beta(a + \mu + \alpha)/[\Lambda(a - b)]$ , the infection will become established and the equilibrium states  $H^* < K$  and  $P^* > 0$  are locally stable. If this condition is not satisfied the parasite fails to persist and  $H^* = K$  and  $P^* = 0$ . *a*, The influence of parasite pathogenicity  $\alpha$  and the natural intrinsic growth rate of the host population  $r$  on the degree of host population depression  $d$ . Parameter values of the model are as follows:  $\beta = 0.01$ ,  $\lambda = 8$ ,  $\mu = 0.1$ ,  $k = 1.0$ ,  $c = 2.5$ ,  $\Lambda = 0.5$ . *b*, The influence of  $\alpha$  and  $r$  on the equilibrium mean parasite burden per host,  $P^*/H^*$ . Parameter values as for *a*.

lations with high reproductive potentials may be achieved by macroparasites with the ability to produce large numbers of transmission stages to ensure the rapid accumulation of parasites within individual hosts.

The most important prediction of both models is related to the use of parasites as biological control agents; direct life-cycle

micro- and macroparasites of low to moderate pathogenicity are the most effective suppressors of host population growth. Very pathogenic organisms are likely to cause their own extinction but not that of their host. These conclusions seem to be reliable as they are derived independently from two mathematical models of very different structure.

Current practice is to select highly pathogenic organisms for the biological control of pest species<sup>1-8</sup>. Such pathogens may cause high initial mortality within the pest population in a manner analogous to a single application of a chemical pesticide. However, in the absence of repeated introductions they are unlikely to achieve maximum and lasting depression of the pest population.

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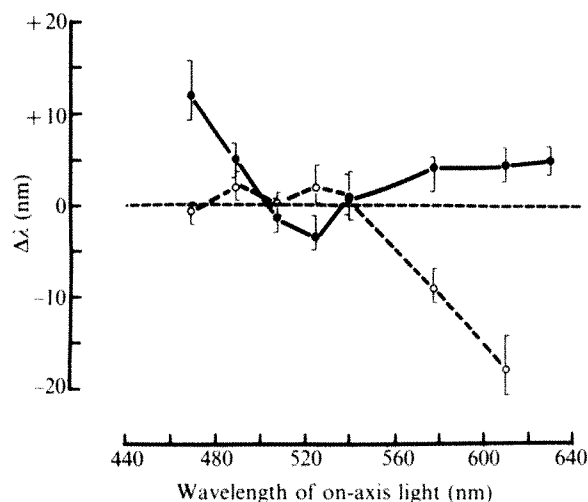
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## The Stiles-Crawford hue shift following photopigment depletion

THE colour of a light is determined by such factors as its dominant wavelength, luminance, colorimetric purity, locus of retinal stimulation, surround chromaticity and preceding adapting field. Stiles and Crawford<sup>1</sup> were first to note that the position at which a small pencil of light enters the pupil also affects the light's colour. In general, light that enters through the centre of the pupil is seen as brighter than the same light impinging on the same retinal area but passing through the periphery of the pupil. This is known as the Stiles-Crawford effect of the first kind (S-C-I). It has been demonstrated that this effect is due to the directional sensitivity of the receptors<sup>2-4</sup> and is much more pronounced in cones than in rods<sup>5-8</sup>. Stiles<sup>9</sup> also showed that two monochromatic lights of the same wavelength, one passing through the centre of the pupil (on-axis), the other entering through the periphery (off-axis), will differ in hue even after they are equated for brightness. (There is also a small change in





**Fig. 1** S-C II for subject BW.  $\Delta\lambda$  represents the shift required to bring an off-axis light to the same wavelength as an on-axis light after both lights have been matched in hue and brightness. Circles are means of two or three separately determined points of subjective equality. Vertical lines are the 5–95% range estimated from the psychometric functions used in deriving the means. ●, No-bleach condition; ○, high-bleach condition. The horizontal dashed line is the predicted result for the high-bleach condition based on the self-screening model.

saturation that becomes almost negligible at the long-wavelength end of the spectrum.) This is known as the Stiles-Crawford effect of the second kind (S-C II). For example, to match in hue an on-axis light of 610 nm an observer might require an off-axis light of 605 nm. The direction and magnitude of this hue shift varies with the wavelength of the on-axis light (Fig. 1). We have measured S-C II in conditions of varying levels of bleached photopigment. An explanation of the effect based on the absorbance of the photopigment can account for much but not all of the data. For the long-wavelength end of the spectrum, when the photopigment is significantly depleted, some other factor must have a role in mediating this effect.

Related to S-C II is the finding that the proportion of mixed red and green light required to match a yellow light (a metameric match) is different depending on where in the pupil the lights enter<sup>10</sup>. This upset in the metameric match must reflect the change in the shape of the spectral sensitivity curve of at least one of the three cone mechanisms when the light-entry position is changed. An altered shape in the spectral sensitivity curve of at least one of the cone systems can also account for S-C II.

Two hypotheses, that differ in their model of how light interacts with photoreceptors, have been proposed to explain these wavelength-dependent Stiles-Crawford effects. In so doing, these hypotheses have addressed the question of what it is that determines the spectral sensitivity of the cones, which is the fundamental property of the visual system on which our colour perception is based.

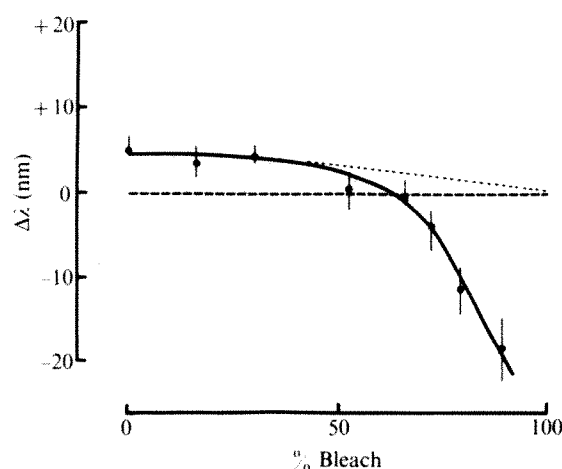
The waveguide hypothesis<sup>11–13</sup>, based in part on physical optics, proposes that as the diameters of the photoreceptors are only slightly greater than the wavelength of light, the receptors act as dielectric (optical) waveguides. Light is propagated through and around an optical waveguide in non-uniform patterns of energy known as modal patterns. The amount of light travelling inside the guide is given in part by the kind of modal pattern produced. Also the amount of light available for absorption by the photopigment varies with the wavelength of the incident light and the angle of incidence. These, of course, are the two independent variables associated with S-C II. It is possible to derive a theoretical curve that closely matches the one seen connecting the filled circles in Fig. 1 by varying the parameters of the waveguide that determine its sensitivity (for example, refractive indices of the receptor and its surrounding medium, outer segment diameter, length and shape)<sup>14,15</sup>.

According to the self-screening hypothesis<sup>16–18</sup>, which is based solely on geometric optics, light that enters through the pupil off-axis passes through less photopigment than on-axis light because of the former's oblique angle of incidence at the receptor layer. Such a shortening of the pathlength that the light takes through the pigment is equivalent to a reduction in the absorbance of the pigment. This follows from the Beer-Lambert law as does the fact that such a reduction has the effect of narrowing the spectral sensitivity curve of the cone system(s) involved. Self-screening has been shown to provide a reasonably good quantitative account of S-C II, if it is assumed that the absorbance of the photopigment(s) is about 0.5 or greater<sup>18</sup>. Based on direct<sup>19,20</sup> and indirect<sup>17</sup> estimates of the absorbance of human cone photopigments coupled with the assumption that passing light through the periphery of the pupil effectively shortens the pathlength, self-screening must to some extent be occurring and therefore must contribute at least in part to S-C II. Furthermore, self-screening provides a model with fewer assumptions and fewer free parameters than the waveguide model. We thus decided to test the generality of the self-screening model to see if S-C II could be accounted for entirely in terms of geometric optics and the absorption properties of photopigments.

In accord with the Beer-Lambert law, the lower the initial absorbance of a pigment, the smaller will be the range over which the absorbance can further be reduced. Thus, inherent in the self-screening model is the prediction that the magnitude of S-C II will be directly proportional to the initial absorbance of the photopigment. In the extreme case, when nearly all the photopigment is depleted, no measurable effect should be obtained.

It is possible to manipulate the initial absorbance of the photopigment by exposing the eye to an adapting light of a certain intensity. Such a bleaching light renders a given amount of pigment incapable of any further absorption of light. The reduction in concentration of available quantum-catching pigment thereby reduces the absorbance of the photopigment.

In our experiment, we measured S-C II in a no-bleach and a high-bleach condition in three subjects. Each subject was presented with two juxtaposed, circular fields each 20' in visual angle. The stimuli were presented in maxwellian view to one eye and were seen foveally. The source images, focused in the plane of the pupil, were 1 mm in diameter. One field, the standard, was



**Fig. 2** S-C II at 610 nm as a function of the level of bleached photopigment for subject BW. The points are derived as described in Fig. 1, except that each point represents the result from a single session. Lightly dashed line shows the predicted result for bleaching levels above 40% based on the self-screening model. Below 40% the self-screening prediction and the obtained results coincide. Predictions are based on the receptor primaries of Vos and Walraven<sup>24</sup> assuming an absorbance of 0.5 for each pigment. It is also assumed that both pigments are bleached at the same rate by the white adapting field.

fixed in wavelength for a given session and entered through the pupil on-axis. The other field was varied around the standard in steps of 2 nm and entered through the pupil off-axis. (The position in the pupil that yields the peak luminous efficiency of a light is not always the centre. Therefore, we measured S-C I along the horizontal meridian to determine the pupillary position for each subject that represented the peak of his S-C I curve. For all subjects, the peak occurred within 1 mm of the geometric centre of the pupil. This was termed the on-axis condition. The off-axis condition was achieved by displacing the source image 3 mm to the opposite side of the pupil along the horizontal meridian.) The subject was required to state whether the variable field appeared to be a hue associated with a wavelength longer or shorter than the wavelength associated with the hue of the standard. Before each judgment, the subject matched the two fields in brightness. Residual differences in saturation, which were for the most part small, were disregarded. The luminances of the test fields were set by the subject to the lowest level possible in making a perfect match. Each wavelength chosen for the variable field was repeated five times. From the resulting psychometric function, the point of subjective equality could be determined. This was done for eight standards: 470, 490, 508, 525, 540, 578, 610 and 630 nm. All lights, except the white adapting field, were monochromatic (Bausch and Lomb grating monochromators, focal length, 0.5 m) with half-band widths of 3 nm. Each standard was repeated in a second session. If the results from the first and second session differed by more than 3 nm, a third session was included. For the high-bleach condition, following a 4-min exposure to a 5°, xenon-white adapting field that was calculated to bleach 88% of the mid- and long-wavelength sensitive cone pigments, each subject was presented with the following sequence of stimuli: a 2-s dark interval, a 1-s exposure of the test fields, a 10-s presentation of the bleaching field. This cycle, which maintained the percentage of pigment bleached at a constant level, continued for the duration of the session (about 0.5 h). The percentage of pigment bleached was calculated from Rushton and Henry's reflection densitometry measurements and pigment kinetic equations<sup>21</sup>. For the no-bleach condition, the subjects dark-adapted for 10 min and were then presented the test fields for 1 s every 4 s.

Figure 1 (filled circles) shows results for one subject for the no-bleach condition and are similar to the findings of others<sup>9,16,18</sup>. The other subjects' data agree with those shown here. For example, when an on-axis light is set to 610 nm, the subject requires that an off-axis beam of light be approximately 605 nm for the two lights to match. Figure 1 (unfilled circles) are the results for the high-bleach condition. For this condition, the self-screening prediction (no hue shift) is represented by the horizontal, dashed line. While the data roughly agree with the prediction up to 540 nm, at longer wavelengths the empirical findings depart significantly from the theoretical curve. With 610 nm as an example again, in the no-bleach condition, an off-axis light of this wavelength appeared too red compared with an on-axis light of the same wavelength. In the high-bleach condition, a 610-nm, off-axis light appeared far too yellow when compared with a 610-nm, on-axis light.

We examined 610 nm at intermediate bleaching levels (Fig. 2). Self-screening predicts that as the percentage of bleached pigment increases, the magnitude of the effect should decrease until, finally, at close to a 100% bleach, the effect should be unmeasurable. The data are in agreement with the self-screening prediction for low and intermediate bleaching levels, but, above a 65% bleach, theory and data are discrepant. Our results for the long-wavelength end of the spectrum suggest that at high bleaching levels a factor other than self-screening, such as waveguide effects, must be invoked to account for S-C II. This is consistent with Weale's<sup>22</sup> conclusion that long-wavelength light is treated preferentially in terms of the physical optics of the receptors. It might be that lights used to bleach a substantial portion of photopigment alter receptor properties in such a way as to increase a waveguide effect.

In conclusion, the present data indicate that a self-screening model can adequately account for S-C II when the eye is adapted to lights that bleach up to about 65% of the photopigment. This means that in such light-adapting conditions, the spectral sensitivity of each of the individual cone mechanisms is determined largely or solely by the absorption characteristics of their respective photopigments and the geometric optical properties of the receptors. However, when the photopigment is substantially depleted, possible waveguide effects of the receptors are revealed and have a significant role in determining spectral sensitivity.

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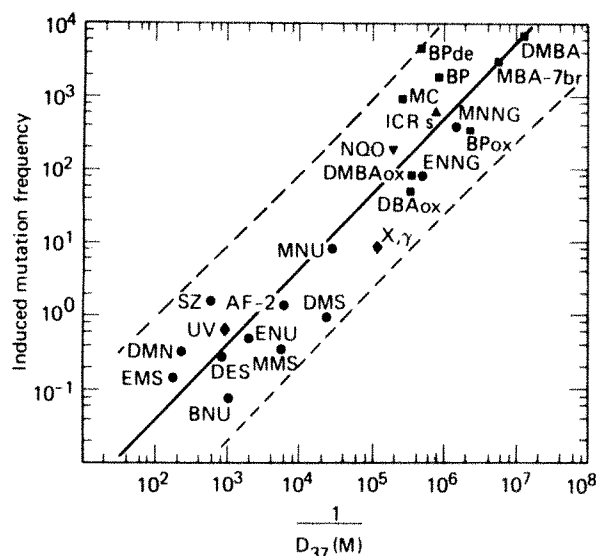
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## Estimating maximum limits to mutagenic potency from cytotoxic potency

RAPID and reliable screening methods are required for identifying environmental mutagens and estimating their mutagenic potency in preparation for use of more elaborate tests to assess the genetic risk to man. Many compounds are effectively screened by *in vitro* mutagenesis assays using either bacterial or mammalian cells. Although tests with mammalian cells are not as rapid and inexpensive as microbial assays, they are needed to confirm and extend the bacterial results, and probably provide a better assessment of potential risk to humans than do microbial tests. However, the screening of all potential mutagens with mammalian cell mutagenicity assays, although desirable, does not seem feasible unless, as we propose, test compounds are first pre-screened on the basis of their cytotoxic potency. On theoretical grounds, one expects a certain minimum cytotoxic potency to correlate with mutagenic potency, particularly when the latter is measured using forward mutations that result in inactive gene products. Specifically, cells cannot divide unless a substantial fraction of their genome is functionally intact; mutagenic agents should thus be obligatory cytotoxic agents, with a given mutagenicity conferring a certain irreducible cyto-



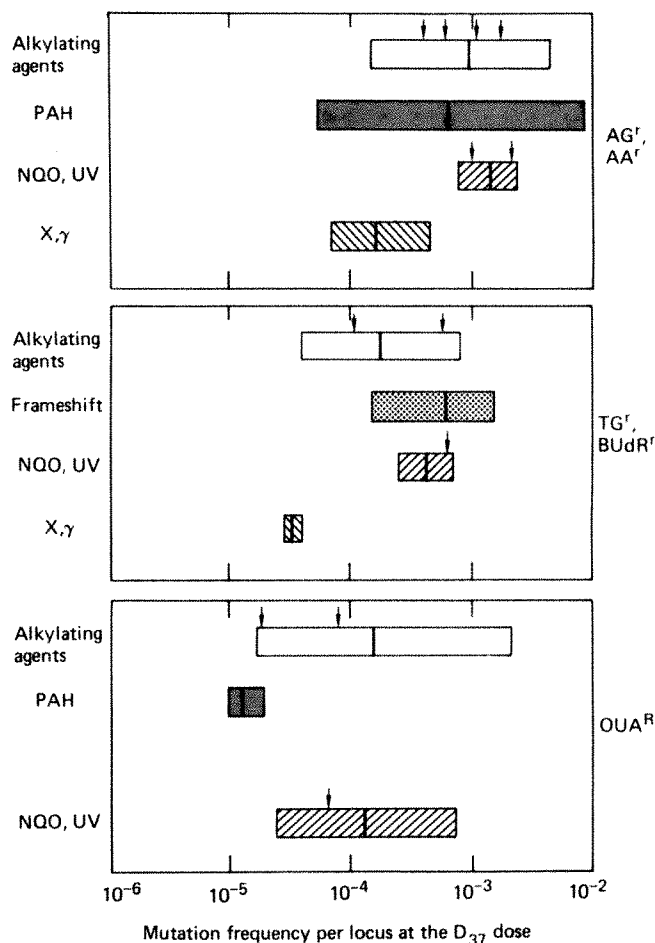
**Fig. 1** The relationship between induced mutation frequency per viable cell (MF) per unit exposure dose (molar) and the reciprocal of  $D_{37}$  (M), that is, the dose required to kill 63% of the initial cell population. Killing is defined as the inability of a cell to undergo continued cell division *in vitro* resulting in a viable cell colony. The  $D_{37}$  values were estimated from (1) authors' precise survival responses; (2) computer fits in ref. 14; (3) published tabular data (occasionally over limited dose ranges). In some cases, the values are approximate but individual estimates generally vary no more than  $\pm 20\%$  and never more than  $\pm 50\%$  (in two cases). Mutation frequency data usually included at least two dose points in the most linear range, except for a few reports having only limited data where a single dose and zero dose (spontaneous frequency) were used. The data included in this plot are those for mutations at the *hprt* locus (hypoxanthine-guanine phosphoribosyltransferase, EC 2.4.2.7), the *aprt* locus (adenine phosphoribosyltransferase, EC 2.4.2.8), and the *tk* locus (thymidine kinase, EC 2.7.1.75). Results in Chinese hamster ovary cells<sup>7-14</sup> ( $n = 25$ ), V79 hamster cells<sup>15-27</sup> ( $n = 18$ ), L5178Y mouse lymphoma cells<sup>28-31</sup> ( $n = 4$ ), normal human diploid fibroblasts<sup>32-38</sup> ( $n = 9$ ) and human lymphoblasts<sup>39,40</sup> ( $n = 4$ ) were combined and expressed as the induced frequency of mutants resistant to azaguanine, AG<sup>r</sup> ( $n = 27$ ); thioguanine, TG<sup>r</sup> ( $n = 27$ ); azaadenine, AA<sup>r</sup> ( $n = 4$ ); bromodeoxyuridine, BUdR<sup>r</sup> ( $n = 2$ ). Abbreviations for the mutagens are given in Table 1. The linear regression line is given by the equation  $\log MF/M = 1.03 (-\log D_{37}) - 3.49$ , with a correlation coefficient of 0.93. The dotted lines represent boundaries of the 95% confidence band for Y data points estimated from X. The physical mutagens were entered on the figure after calculating their potencies relative to EMS, based on the ratios of the number of lesions induced in the DNA of a cell at the  $D_{37}$  doses (UV-induced dimers<sup>41</sup> and total ionisation lesions<sup>5,6</sup>) to the number of alkylations<sup>42</sup> induced by EMS at its  $D_{37}$ . The 'molar equivalent  $D_{37}$ ' values were obtained by multiplying the  $D_{37}$  for EMS ( $6 \times 10^{-3}$  M) by the potency ratios for UV (0.18) and ionising radiation ( $1.4 \times 10^{-3}$ ). The 'molar equivalent mutation frequencies' were obtained by multiplying the geometric means of the observed mutation frequencies per  $D_{37}$  for UV and ionising radiation by the respective molar equivalent  $D_{37}$  values. The calculated parameters were added to the graph, but were not included in the regression calculation. The lower detection limit of the assays (stippled area) was estimated, assuming that (1) at frequencies below  $10^{-5}$  induced mutations per viable cell, statistically significant determinations of MF are generally not practical, and (2) the maximum feasible exposure dose is usually  $\leq 10$ -fold higher than the  $D_{37}$  concentration.

toxicity. We show here that the cytotoxic potency of 22 chemical mutagens is highly correlated with their mutagenic potency as assayed in five rodent and human *in vitro* cell systems. This relationship implies that the maximum potential mutagenic potency of such compounds may be reliably estimated from rapid and straightforward measurements of their cytotoxic potency, the latter defined as the failure of cultured cells to undergo continued cell division. The estimate is necessarily a maximum one, as an agent may exert cytotoxic effects by pathways independent of its mutagenic action.

A correlation between cell survival and mutagenic response has been noted for ionising radiation mutagenesis<sup>1</sup> and also seems to be applicable to ultraviolet radiation and certain chemicals<sup>2-4</sup>. Roberts *et al.* have discussed the molecular aspects of cellular lethality and mutagenesis in terms of damage to DNA<sup>5,6</sup>. To test this relationship further, we have compiled data on the cytotoxicity and mutagenicity of the 24 chemical and physical mutagens listed in Table 1. As the measure of cytotoxic

potency, we have used the  $D_{37}$  unit of survival, defined here as the dose required to kill approximately 63% of the initial cell population. Unlike  $D_0$ , estimation of  $D_{37}$  is usually possible from the limited survival data that accompany published mutation frequencies;  $D_{37}$  generally measures survival within the linear portion of the dose response for mutation (lower survivals are frequently out of the linear mutation range). Moreover, this parameter reflects differences in repair capability or fidelity that may influence both toxicity and mutagenicity.

Figure 1 compares the frequency of forward mutations induced per applied dose with the reciprocal of the molar dose required for  $D_{37}$  survival in three rodent and two human cell systems<sup>5-42</sup>. As plotted, the data constitute a potency index, extending from weakly mutagenic and less toxic compounds to highly mutagenic agents that are toxic at very low concentrations. The relative increase in cytotoxicity is accompanied by a proportional increase in mutagenicity, that is, the slope of the regression relationship is  $1.03 \pm 0.03$ . The observed correlation between cytotoxicity and mutagenicity suggests that a determination of the  $D_{37}$  dose of toxic agents can be used to estimate the maximum potential-induced mutation frequency. In particular, these data permit the hypothesis that no agents will be found whose location on this graph is significantly above the upper dotted line. Whereas mutagenicity and cytotoxicity separately range over six orders of magnitude, no agent can



**Fig. 2** The induced frequency per viable cell (MF) calculated for the  $D_{37}$  exposure dose of the physical and chemical mutagens listed in Table 1. The loci include *hprt* (AG<sup>r</sup>, TG<sup>r</sup>), *aprt* (AA<sup>r</sup>) and *tk* (BUdR<sup>r</sup>), as well as resistance to ouabain (OUA<sup>r</sup>) involving presumed lesions in the Na<sup>+</sup>-K<sup>+</sup>-ATPase enzyme (EC 3.6.1.3). The inner mark within the bars represents the geometric mean of data for all systems, with the range of reported values indicated by the ends of each bar. The arrows refer to the author's data for the Chinese hamster ovary multiple marker system<sup>7</sup>. According to the assumptions listed in Fig. 1, the lower limit for determining the MF at the  $D_{37}$  would be  $10^{-6}$ .



**Table 1** Compounds evaluated for mutagenic potency

Compound	n	Ref.
<b>Alkylating agents</b>		
Streptozotocin (SZ)	1	17
Dimethylnitrosamine (DMN)	2	23, 31
Ethyl methanesulphonate (EMS)	15	7, 11–13, 15, 16, 26, 28, 29, 35, 42
Diethylsulphonate (DES)	1	10
N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	13	7, 9, 13, 17, 18, 21, 25, 27, 34, 39, 40
N-methyl-N-nitrosourea (MNU)	2	9, 40
N-ethyl-N-nitrosourea (ENU)	1	9
2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2)	2	27, 36
N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG)	1	9
N-butyl-N-nitrosourea (BNU)	1	9
Methyl methanesulphonate (MMS)	2	10, 30
Dimethylsulphate (DMS)	1	10
<b>Polycyclic aromatic hydrocarbons (PAH)</b>		
Benzo(a)pyrene, diol epoxide (BPde)	1	24
3-Methylcholanthrene (MC)	2	22
Benzo(a)pyrene (BP)	3	20, 22
7,12-Dimethylbenz(a)anthracene (DMBA)	2	22
7-Bromomethylbenz(a)anthracene (MBA-7br)	1	19
7,12-Dimethylbenz(a)anthracene, 5,6-oxide (DMBAox)	1	37
Dibenz(a,h)anthracene, 5,6-oxide (DBAox)	1	37
Benzo(a)pyrene, 4,5-oxide (BPox)	1	37
<b>Frameshift mutagens</b>		
Heterocyclic nitrogen mustard compounds (ICRs)	5	14, 40
<b>Physical mutagens</b>		
X and $\gamma$ rays	4	2, 6, 8, 32, 33
Ultraviolet irradiation (UV)	6	11, 13, 16, 26, 38, 41
<b>Miscellaneous</b>		
4-Nitroquinoline-1-oxide (NQO)	4	7

n, Number of determinations evaluated for each compound (some at multiple genetic loci).

induce more than approximately one forward mutation at a given locus per 100 cells surviving at the D<sub>37</sub> dose. If this hypothesis is valid, this is a biological limit to mutagenic potency that is demonstrated by compounds whose cytotoxicity is due solely to mutational events.

On the other hand, we believe the absence of points below the lower dotted line to be artefactual, representing the bias of reported mutagenesis studies for compounds whose ratio of mutagenicity to cytotoxicity is relatively high. There are compounds which would graph somewhere in this lower range; for example, a few derivatives of heterocyclic nitrogen mustard frameshift mutagens<sup>14</sup>, a metabolite of a polycyclic hydrocarbon<sup>37</sup>, and an inhibitor of DNA synthesis<sup>43</sup> are highly toxic, but are apparently non-mutagenic in mammalian *in vitro* test systems. Also, certain chemicals yield multi-phasic cell survival curves that cast doubt on the validity of the D<sub>37</sub> concept for these mutagens. The shaded area in Fig. 1 reflects values below the detection limit for the systems studied. The cytotoxicity data for non-mutagens would also fall within this range. Thus, the dynamic range for forward mutations in mammalian *in vitro* systems is approximately four orders of magnitude (from the lowest detectable level to the highest possible mutagenic potency at the D<sub>37</sub> level of cytotoxicity). Compounds near the bottom of this range will presumably be of greater concern as cytotoxic agents than as mutagens.

The relative mutagenicity of different compounds at a given cytotoxicity (Fig. 1) is not significantly related to the class of mutagen or the forward mutation marker scored. This is shown in Fig. 2, which displays the observed values of the mutation frequency per locus at the D<sub>37</sub> dose for different classes of mutagens at different genetic loci. This variation is also not due to systematic differences among cell systems (data not shown).

Cytotoxicity, as defined (Fig. 1), may result from genetic (DNA-related) and non-genetic mechanisms of injury. Agents that induce the highest mutation frequencies at the D<sub>37</sub> dose may have the tightest coupling between genetic injury and lethality, that is, the cytotoxicity related to DNA damage pre-

dominates over other forms of general toxicity. Agents inducing lower mutation frequencies at the D<sub>37</sub> dose may produce a larger proportion of non-genetic injury.

The remarkable similarity among mutation frequencies at equitoxic concentrations has been noted by others for several mutagens<sup>5,6,17,37</sup>. The correlation between cytotoxicity and mutagenicity need not be due solely to the same type of cellular damage causing both lethal and mutational events<sup>4</sup>; factors that influence the effective exposure of the cells to chemical mutagens (toxification, detoxification) may affect both end points proportionally.

Additional data are needed to establish that partitioning of genetic and non-genetic toxicity induced by a mutagenic agent can be estimated from the magnitude of mutation induced at the D<sub>37</sub> dose. However, the data in Fig. 1 indicate that cytotoxicity as measured by the D<sub>37</sub> dosage provides a quantitative estimate of potential mutagenicity. Where appropriate, more specific bioassays can then be applied to determine precisely the *in vitro* mutagenic potency.

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## Hyperdiploid species hybrids for gene mapping in *Xenopus*

SPONTANEOUS endo-reduplication in the female germ line of hybrids has made it possible to produce several strains of isogenic *Xenopus*, providing one of the many features required of a laboratory animal<sup>1,2</sup>. The usefulness of *Xenopus* in the laboratory, however, would be improved if a gene map were available. We report here that hybrids can also be used to define linkage groups and their assignment to chromosomes. Our method is based on the possibility of creating aneuploid *Xenopus* which are diploid for the chromosomes of one species but have supernumerary chromosomes of a second species<sup>3</sup>. This method complements those already available<sup>4-8</sup>, and has the advantage that because traits can be monitored in the whole animal, a large repertoire of markers will be made available.

Figure 1 is a schematic presentation of how such animals are obtained, in this case for *X. laevis* × *X. gilli* hybrids. First generation hybrid females from this and other species combinations frequently produce diploid eggs from allotetraploid oocytes produced by endo-reduplication in the germ line. These oocytes have a complete set of bivalents from each parent species, synapsis being restricted to endo-reduplicated identical chromosomes<sup>1,9</sup>. Fertilised by sperm of one of the parent species, such eggs develop into triploid females (WZZ) because the female determinant W is dominant<sup>10</sup>. Triploid oocytes of such backcross females show a diploid number of bivalents, presumably the two homologous sets of chromosomes, with the third genome consisting of univalents. No trivalents have been observed<sup>9</sup>. The eggs thus contain a complete haploid chromosome set of the backcross species, *X. laevis* in our example, and, on the assumption of a random distribution of univalents, 0–18 chromosomes of the other species. Random distribution is indeed observed in the case of the easily recognisable nucleolus organiser chromosome. Fertilised again by *X. laevis* sperm, hyperdiploid embryos are obtained which are diploid for *X. laevis* but trisomic for a random selection of *X. gilli* chromosomes. Most of these zygotes undergo abnormal embryogenesis and die<sup>3</sup>, but 1–3% of them reach adulthood.

We have analysed 14 such hyperdiploid adults for karyotype and for the expression of *X. gilli*-specific characters. Antisera raised in *X. laevis* (as described elsewhere<sup>11</sup>) enabled us to detect by immunoelectrophoresis up to six *X. gilli*-specific serum antigens (Fig. 2), one of which was the heavy chain of low-molecular weight immunoglobulin (7S, IgG-like). Antiserum B10 was made specific for *X. gilli* Ig by immunoabsorption; A84 also contained antibodies against *X. gilli* Ig. We have not established the homologies between the serum proteins revealed by the different antisera. Two *X. gilli*-specific erythrocyte antigens were distinguished by lysis after iso-electrofocusing of specific antibodies (antisera A38 and A39).

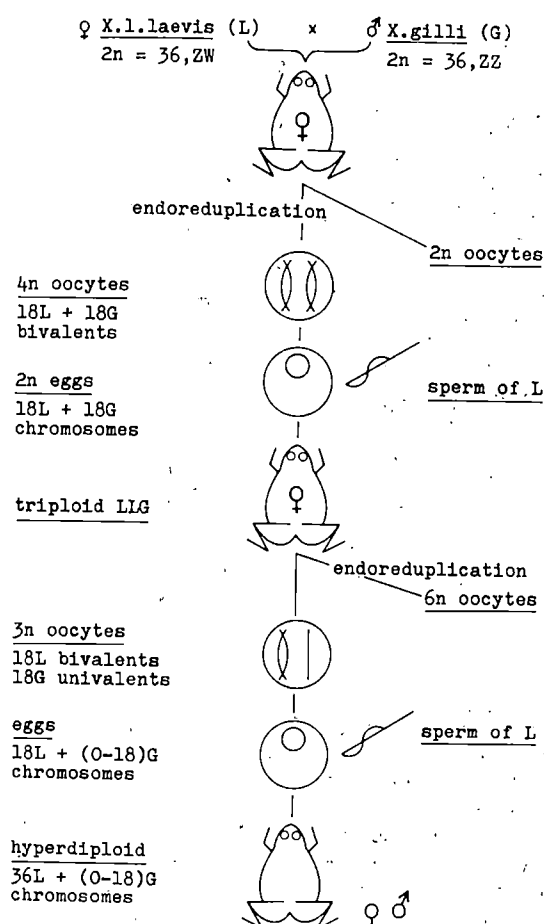


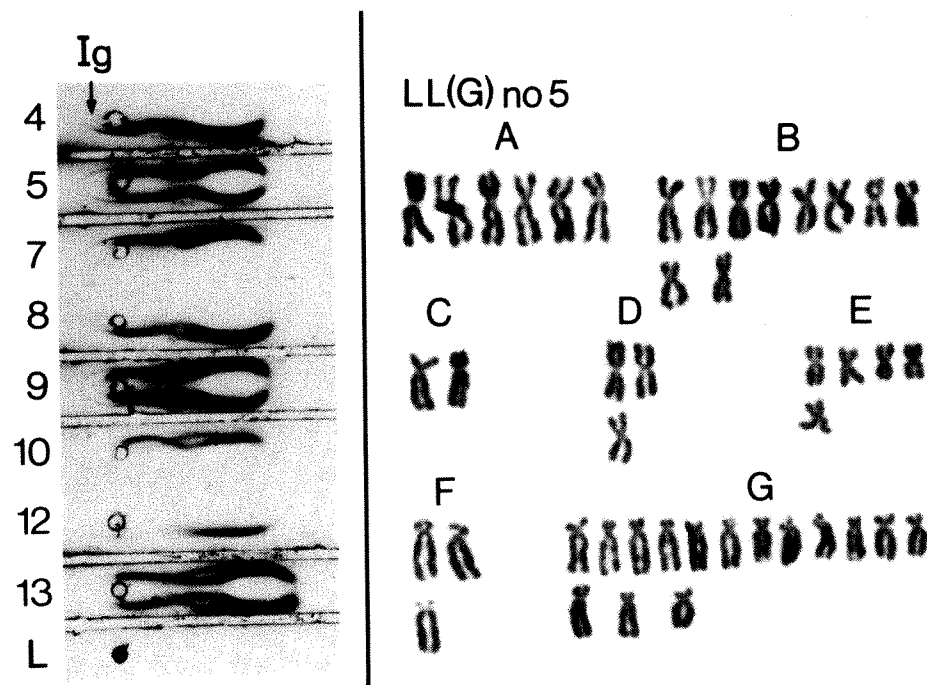
Fig. 1 Schematic presentation of pedigree of hyperdiploid *Xenopus* species hybrids.

Haemagglutination with antiserum A37 revealed an additional *X. gilli* erythrocyte antigen. Table 1 shows that *X. gilli* Ig and the erythrocyte surface antigens segregated as unlinked characters. They were not linked with either the single serum protein revealed by antiserum A85 or with those revealed by antiserum A82. This is in agreement with results obtained on diploid backcross animals of clone LG 15 (ref. 11) where, in contrast to most other LG clones, there was recombination between *X. gilli* and *X. laevis* chromosomes<sup>2</sup>. These characters depend therefore on genes on separate chromosomes. The lack of linkage between erythrocyte surface antigens suggests that some of them may represent minor histocompatibility antigens rather than major ones.

Chromosomes were prepared from phytohaemagglutinin-stimulated leukocyte cultures<sup>12</sup>. The karyotypes of both species are similar ( $2n = 36$ ) except for a chromosome pair with a

Table 1 Number of supernumerary *X. gilli* chromosomes and occurrence of *X. gilli*-specific serum and erythrocyte antigens in a sample of hyperdiploid *Xenopus* hybrids

<i>X. gilli</i> specificities	Control LG	Hyperdiploid LL(G) animal no.													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Number of supernumerary chromosomes	(18)	11	13	16	8	8	8	14	8	8		11	8	8	8
Immunoglobulin heavy chain: Antiserum B10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Number of erythrocyte antigens: Agglutination, antiserum A37	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lysis, antisera A38 + A39	2	2	2	2	2	2	2	2	1	2	2	0	1	1	2
Number of serum proteins: Antiserum A82	3	1	3	3	3	3		3	3	3	3	0	0	3	3
A83	6	6	6	5	5	5		6	6	6	4	0	1	2	4
A84	5	3	5	5	4	4		5	3	5	4		2	5	
A85	1	0	1	1	1	1		1	1	0	0		0	1	



**Fig. 2** Immunoelectrophoretic analysis of the serum of eight hyperdiploid *Xenopus* hybrids with the antiserum A84. Serum samples in the wells from LL(G) animals nos. 4–13, and *X. laevis* (L) control. Antiserum A84 in the trough. Cathode to the left. Migration conditions: 1 h, pH 8.6, 5 mA per slide. Note the expression of only two *X. gilli* antigens, one of which is immunoglobulin (Ig, heavy chain) by animal no. 12, and 3 to 5 antigens by the other specimens. *X. laevis* control is negative. The karyotype of animal no. 5 shows groups (A–G) of similar chromosomes; supernumerary chromosomes on a second line.

secondary constriction specific for *X. gilli*<sup>13</sup>. The chromosomes can be classified into seven groups (Fig. 2) on the basis of relative length and arm ratio<sup>6</sup>, but individual chromosome recognition requires differential staining. Whereas the total number of supernumerary chromosomes can be determined (Table 1), their assignment to groups remains uncertain. Nevertheless, the nucleolus organiser chromosome (group F) is easily recognised in both mitotic and interphase cells and can be excluded as a carrier of one of the characters checked so far. Acrocentric chromosomes can also be counted. Animals 9, 13 and 14 had only two supernumerary acrocentric chromosomes; thus, not more than two of the characters analysed may depend on group G chromosomes. The four specimens which were not trisomic for the chromosome of group D were also deficient for one of the *X. gilli* erythrocyte antigens, indicating the possible chromosome location of the corresponding gene.

Some animals had a hypertriploid constitution due to failure of second meiotic division. Because the second meiotic division segregates chromatids, eggs which retain the second polar body contain a diploid set of *X. laevis* chromosomes as well as both chromatids of the *X. gilli* univalents distributed at meiosis I. Zygotes are, therefore, triploid for *X. laevis* and supernumerary for pairs of *X. gilli* chromosomes. Animal 12 had a bimodal chromosome number with 36 and 44 chromosomes. It is not certain whether the karyotype found in a given tissue is representative for the whole animal. Mosaicism could arise after non-disjunction during ontogenesis because the original zygotic constitution might be improved through the loss of supernumerary chromosomes.

It is striking that in spite of the variable degree of hyperdiploidy, most animals had most of the *X. gilli* characters studied. Was this attributable to redundancy of genes? Because several levels of ploidy occur in *Xenopus*<sup>14</sup>, one might consider both *X. laevis* and *X. gilli* to be ancient tetraploids. Even a restricted number of supernumerary chromosomes could then contain one of the originally duplicated genes. This would also imply that activity of duplicated genes has been conserved during evolution and that our methods do not distinguish between the various alleles. It seems more likely that survival of aneuploids is better when animals are functionally hypotriploid rather than trisomic for only a few chromosomes.

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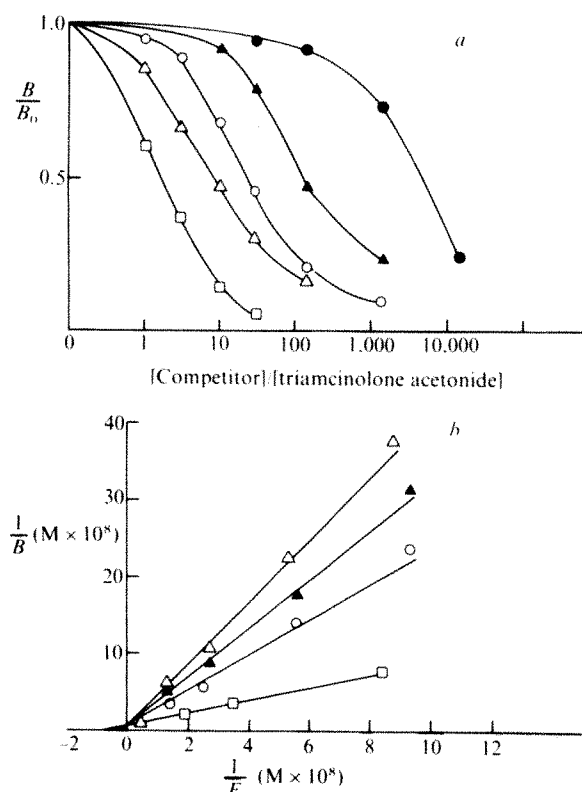
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## 17 $\beta$ -Carboxamide steroids are a new class of glucocorticoid antagonists

GLUCOCORTICOID hormones exert many of their effects by modifying the activity of certain enzymes in target tissues, probably as a result of alterations in gene expression<sup>1</sup>. Experimental models have been developed in which enzyme induction by various steroids correlates well with their anti-inflammatory, thymolytic and glycopeptic properties. This is the case for tyrosine transaminase in cultured rat hepatoma (HTC) cells<sup>2,3</sup>. Studies in this system showed that, in addition to glucocorticoid agonists, partial agonists and antagonists could be identified<sup>4</sup>. The latter compete with agonists for binding to the intracellular glucocorticoid receptor but do not trigger the glucocorticoid effect<sup>5</sup>. Compounds which specifically antagonise glucocorticoid action at the target cell would be useful for the treatment of certain diseases characterised by excessive production of glucocorticoids<sup>1</sup> and for further studying the molecular mechanism of action of these hormones. However, unlike anti-androgens and anti-oestrogens<sup>6</sup>, the only known glucocorticoid antagonists are natural steroid hormones (such as progesterone and testosterone) or their analogues, which interact with their own receptors and plasma binding proteins and are therefore not very





**Fig. 1** Glucocorticoid receptor binding of 17 $\beta$ -carboxamide derivatives of dexamethasone. Livers from adrenalectomised male rats (Wistar, 250 g) were homogenised in buffer containing 20 mM Tris, 20 mM 2-mercaptoethanol, 3 mM MgCl<sub>2</sub> and 20% glycerol, pH 7.5. The homogenate was centrifuged at 105,000g for 2 h to yield the cytosol. Cytosol aliquots were incubated with [1,2,4(*n*)-<sup>3</sup>H]triamcinolone acetonide (28 Ci mmol<sup>-1</sup>, Radiochemical Centre). After 2 h at 0°C, specific binding to the glucocorticoid receptor was determined in duplicate by charcoal adsorption assay<sup>5</sup>. *a*, Inhibition of binding of 35 nM triamcinolone acetonide by increasing concentrations of dexamethasone and four of its derivatives (for nomenclature, see Table 1). Ordinate: ratio of binding of <sup>3</sup>H-labelled triamcinolone acetonide in the presence of competitor (*B*) to binding in the absence of competitor (*B*<sub>0</sub>). Abscissa: concentration ratios. □, Dexamethasone; Δ, DXB; ○, DXP; ▲, DXN; ●, DXO. *b*, Double-reciprocal plot of the specific binding (*B*) of triamcinolone acetonide at various concentrations (*F*), in the absence of competitor and the presence of a constant concentration of competitors. Δ, 0.25 μM DXB; ▲, 1 μM DXN; ○, 0.5 μM DXP; □, no competitor.

specific. We report here that new derivatives of dexamethasone, a specific glucocorticoid agonist which does not bind to rat or human plasma transcortin<sup>5</sup>, can block the induction of tyrosine transaminase in HTC cells.

The novel steroids listed in Table 1 were synthesised and purified in our laboratory (P.F. and P.L., in preparation). They include the 17 $\beta$ -carboxylic steroids obtained by periodic oxidation of cortisol, corticosterone and dexamethasone, respectively, and seven 17 $\beta$ -carboxamide derivatives. The possible interaction of these steroids with the glucocorticoid receptor was studied in liver cytosol from adrenalectomised rats, using as specific ligands <sup>3</sup>H-labelled dexamethasone or triamcinolone acetonide, both synthetic agonists. Even at a 1,000-fold excess the 17 $\beta$ -carboxylic compounds DCAC, HCAC and DXO (see Table 1 for structures) barely inhibited binding of the <sup>3</sup>H-labelled steroids. However, after coupling the 17 $\beta$ -carboxyl group of DXO with various amines, clear inhibition by these dexamethasone derivatives was observed (Fig. 1*a*). The benzylamine-substituted compound DXB was the most active, a 10-fold excess being sufficient to produce a 50% reduction in the binding of the potent glucocorticoid, triamcinolone acetonide (Fig. 1*a*). This phenomenon was further investigated using different concentrations of radioactive ligand. Double-reciprocal plots suggested that the 17 $\beta$ -carboxamide derivatives

inhibited binding of triamcinolone acetonide to a homogeneous population of receptor sites in a competitive manner. Representative experiments are shown in Fig. 1*b*. The order of affinity was DXB > DXH > DMP > DXP > DXM > DXN > DCACP > DXO, with an equilibrium dissociation constant for DXB (0.8–1.1 × 10<sup>-7</sup> M) as high as that of corticosterone, the natural glucocorticoid in rats.

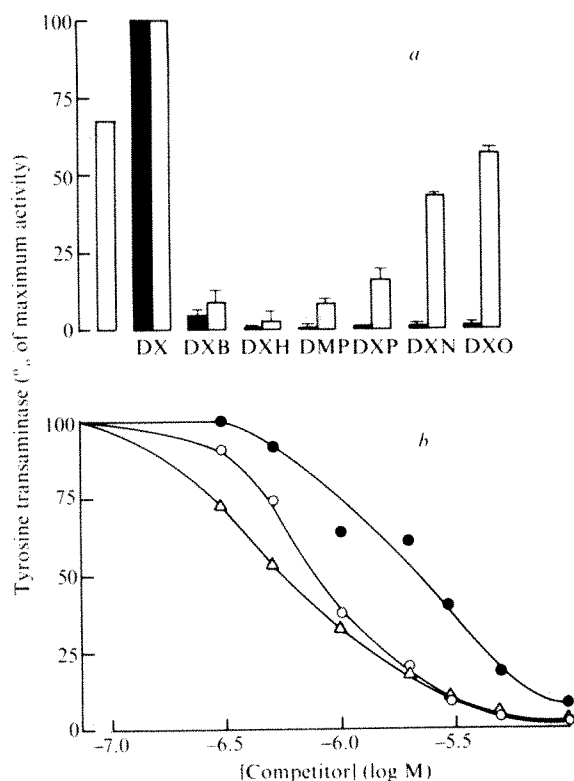
One might therefore predict that some of the dexamethasone derivatives would mimic the action of dexamethasone on tyrosine transaminase activity in HTC cells. Surprisingly, in conditions where dexamethasone produced a 10–15-fold increase of tyrosine transaminase activity, neither of the 17 $\beta$ -carboxamide steroids at concentrations up to 10 μM significantly modified the baseline activity of this enzyme (shaded bars in Fig. 2*a*). However, when assayed for receptor binding in HTC cell cytosol, the synthetic steroids competed with <sup>3</sup>H-labelled dexamethasone as they did in rat liver extracts. The order of affinity was the same. Tyrosine transaminase induction experiments were therefore carried out in which HTC cells were incubated with mixtures of dexamethasone and 17 $\beta$ -carboxamide steroids. We found that, although devoid of intrinsic glucocorticoid activity, DXH and DMP could inhibit the stimulating effect of dexamethasone on tyrosine transaminase by more than 85% (open bars, Fig. 2*a*). DXB was slightly less inhibitory than DXH because it had a very small agonist activity of its own. The other steroids also antagonised the glucocorticoid effect, in keeping with their affinity for the HTC receptor (Fig. 2*a*). The latter observation suggests that the inhibition of tyrosine transaminase is not a nonspecific action of high concentrations of the dexamethasone derivatives. Data in Fig. 2*b* support this interpretation, as it shows that the anti-glucocorticoid effect is dose dependent and that the order of potency of the inhibitors is consistent with their affinity for the HTC receptor.

Unlike natural corticosteroids, their 9 $\alpha$ -fluoro-derivatives do not bind to transcortin, the plasma corticosteroid-binding globulin. We examined whether this difference was maintained after carrying out the 17 $\beta$ -substitutions described here. Plasma

**Table 1** Natural and synthetic steroids used in this study

Compound*	1-ene	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>
Cortisol	—	H	H	OH	CH <sub>2</sub> OH
DCAC	—	H	H	OH	OH
DCACP	—	H	H	OH	NH-(CH <sub>2</sub> ) <sub>2</sub> -CH <sub>3</sub>
Corticosterone	—	H	H	H	CH <sub>2</sub> OH
HCAC	—	H	H	H	OH
Dexamethasone	+	F	CH <sub>3</sub>	OH	CH <sub>2</sub> OH
DXO	+	F	CH <sub>3</sub>	OH	OH
DXM	+	F	CH <sub>3</sub>	OH	NH-CH <sub>3</sub>
DXP	+	F	CH <sub>3</sub>	OH	NH-(CH <sub>2</sub> ) <sub>2</sub> -CH <sub>3</sub>
DXN	+	F	CH <sub>3</sub>	OH	NH-(CH <sub>2</sub> ) <sub>2</sub> -NH-(CH <sub>2</sub> ) <sub>2</sub> -CH <sub>3</sub>
DXH	+	F	CH <sub>3</sub>	OH	NH-(CH <sub>2</sub> ) <sub>2</sub> -CH <sub>3</sub>
DXB	+	F	CH <sub>3</sub>	OH	NH-CH <sub>2</sub> -C <sub>6</sub> H <sub>5</sub>
Desoximethasone	+	F	CH <sub>3</sub>	H	CH <sub>2</sub> OH
DMP	+	F	CH <sub>3</sub>	H	NH-(CH <sub>2</sub> ) <sub>2</sub> -CH <sub>3</sub>
Triamcinolone	+	F	OH	OH	CH <sub>2</sub> OH

\* Trivial names and abbreviations used in text and figures.



**Fig. 2** Anti-glucocorticoid activity of 17 $\beta$ -carboxamide derivatives of dexamethasone (DX). Rat hepatoma tissue culture (HTC) cells were resuspended in serum-free medium containing 0.1% (w/v) bovine serum albumin and incubated at 37 °C for 16 h in the absence of steroid or in the presence of 10  $\mu$ M dexamethasone to determine, as described previously<sup>4</sup>, the maximum tyrosine transaminase activity of the culture. The latter was 11.4–23.7 mU per mg protein, depending on the experiment, whereas it was 1.7–2.3 mU per mg protein in untreated cultures. Parallel cultures were also exposed to the other steroids, alone or in combination (for nomenclature, see Table 1). *a*, Shaded bars: cultures exposed to 10  $\mu$ M of a single steroid, as indicated. Open bars: cultures exposed to 25 nM dexamethasone alone (first bar on the left) or in combination with 10  $\mu$ M of the steroid indicated. Values given are the range obtained from two to four experiments. *b*, Effect of varying concentrations of the three most potent 17 $\beta$ -carboxamide derivatives on tyrosine transaminase activity in cultures exposed to 25 nM dexamethasone.  $\Delta$ , DXB;  $\circ$ , DXH;  $\bullet$ , DMP.

from adrenalectomised rats was incubated with <sup>3</sup>H-labelled corticosterone and binding to transcortin determined as described elsewhere<sup>5</sup>. We found that a 1,250-fold excess of HCAC, DCAC and DCACP inhibited corticosterone binding by 94, 77 and 71%, respectively, whereas the 9 $\alpha$ -fluoro-derivatives had either no effect or inhibited the binding by only 6–9% (DXB, DXN).

Our results show that glucocorticoid antagonists which bind to the receptor with high affinity can be derived from the synthetic agonists dexamethasone and desoximethasone. Some of the binding properties of these novel steroids are consistent with structure–activity relationships previously defined for the HTC cell glucocorticoid receptor<sup>7</sup>. First, the data confirm the importance of the steroid side chain in assigning both the potency (affinity for the receptor) and the activity class (agonist or antagonist). We now find that the loss of potency on removal of the carbon atom in position 21 can be partially restored by coupling various amines at this position. Crystallographic data (M. Devos, P.F. and P.L., unpublished) make it unlikely that the nitrogen atom could substitute for the oxygen atom of the 21-hydroxyl group as an acceptor in a putative hydrogen bond with the receptor. Rather, binding of the 17 $\beta$ -carboxamide

steroids would involve the hydrophobic interaction of the new side chain with the receptor site, as the affinity increases with increasing hydrophobicity of the amine. However, other substituents on the steroid skeleton retain their typical influence on the binding: DCACP and DXP have a potency ratio similar to that of the parent compounds cortisol and dexamethasone. Also, the fact that DMP has a greater affinity than DXP shows that the 17 $\alpha$ -hydroxyl group has the same unfavourable effect on the binding to the HTC receptor in 17 $\beta$ -carboxamide derivatives as in the pregnene series<sup>7</sup>. The persistence of this effect in the absence of a C-21 carbon atom suggests that it could result from a specific influence of the 17 $\alpha$ -hydroxyl group on the orientation of the C<sub>20</sub> ketone as suggested earlier<sup>8</sup>. Finally, our results show that conversion of an agonist into an antagonist is compatible with modifications (1–2 unsaturation; 9 $\alpha$ -fluor-, 16 $\alpha$ -methyl- and 11 $\beta$ -hydroxyl-substitutions) which promote high affinity for the receptor and/or prevent the steroid from binding to plasma transcortin and to cellular proteins other than the receptor. This has important implications for the development of more potent anti-glucocorticoids and receptor-specific ligands possessing chemically reactive functions which would be very useful for receptor purification by affinity chromatography.

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## Guinea pig prostate is a rich source of nerve growth factor

NERVE GROWTH FACTOR (NGF) is essential for the development of sympathetic nerve cells<sup>1</sup>. The richest known source of NGF is the submaxillary gland of the adult male mouse<sup>2</sup>, and NGF from this source has been completely purified and thoroughly characterised<sup>3</sup>. The active entity ( $\beta$ -subunit) is a protein comprising a dimer of identical 118-residue chains<sup>4,5</sup>. Another form of mouse NGF, the 2.5S form, has been isolated<sup>6</sup> and shown to differ from the  $\beta$  entity in that some of the

monomeric chains have undergone enzymatic cleavage of the amino-terminal octapeptide and carboxy-terminal arginine residues<sup>3,4,7-9</sup>. The 2.5S dimer therefore contains both 118-residue chains and shortened chains. The salivary gland of the mouse seems to be unique in that the corresponding glands of the rat, guinea pig, cow, pig, rabbit and man contain no NGF<sup>10,11</sup>. No other clearly characterised source of NGF in mammalian tissue *in vivo* has been reported. We now report a new, rich source of NGF—the prostate glands of the adult male guinea pig.

NGF activity in soluble extracts of guinea pig prostate glands (dorsal and ventral lobes) was measured using a biological assay<sup>12-14</sup> in which the NGF content of a tissue is assessed by the ability of an extract to promote the outgrowth of nerve fibres from explants of dorsal root ganglia from 8-d chick embryos cultured for 24 h in specified conditions. The assay gives the NGF concentration in terms of a biological unit (BU), defined as the amount of NGF required to produce optimum fibre outgrowth. Assuming the same specific activity for guinea pig prostate and mouse submaxillary NGFs, the guinea pig gland is found to contain  $1.8 \pm 0.5$  mg NGF per g wet weight (mean  $\pm$  s.e.m., range 340–9,925  $\mu$ g,  $n = 22$ ). This organ therefore seems to be of similar potency to the mouse submaxillary gland. The levels in the latter organ (0.3 to 5 mg NGF per g wet weight) are known to vary according to the strain of mouse<sup>15</sup> and the extraction procedure.

Both whole antiserum and antibodies purified by affinity chromatography<sup>16</sup> against mouse submaxillary gland NGF were able to inhibit completely the biological activity of the prostate extracts in tissue culture (see ref. 17). The amounts of pure antibody required to block 1 BU ml<sup>-1</sup> *in vitro* of purified mouse NGF and crude extracts of prostate glands were not significantly different (0.62–1.25  $\mu$ g ml<sup>-1</sup> and 0.31–0.62  $\mu$ g ml<sup>-1</sup>).

Extracts of guinea pig prostate gland were tested by immunodiffusion against antiserum to purified mouse NGF (Fig. 1), and thereby compared with mouse NGF. The interaction of the precipitin lines shows that there are antibodies that recognise determinants common to both mouse and guinea pig NGFs, but that the mouse antigen has determinants not present in the guinea pig NGF (spur formation). This implies that a substantial proportion (note length and intensity of spurs) of the antibodies recognise mouse but not guinea pig NGF. The common antigenic sites may be related to the biologically effective site(s) of the proteins, the structure of which might be expected to be conserved, while the other part(s) of the molecule may be more variable between species.

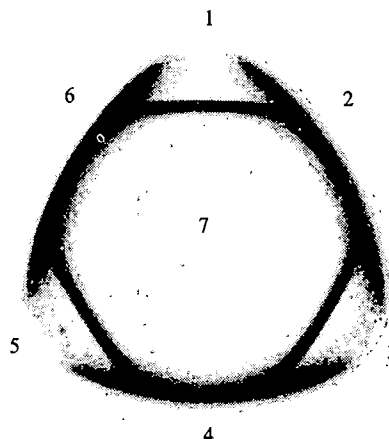
The NGFs were also compared by immunoprecipitation with purified antibodies against mouse NGF, followed by SDS-gel electrophoresis (Fig. 2). Mouse submaxillary glands and guinea pig prostate glands were extracted in the presence of enzyme inhibitors (see Fig. 2 legend) to prevent the enzymatic cleavage of NGF in the crude extracts. In these conditions, most of the mouse NGF remains as the  $\beta$ -subunit (molecular weight of the monomer 13,259, refs 3–5), although a significant proportion exists in the 2.5S form in spite of the presence of enzyme inhibitors. (The SDS gel shows both the 118-residue monomers from the  $\beta$  and the 2.5S forms and the shorter monomers from the 2.5S NGF alone.) The greater intensity of the higher molecular weight band indicates that the  $\beta$  form predominates. In contrast, the guinea pig NGF shows a single band in the same position as that of the 118-residue monomer from mouse NGF. Omission of enzyme inhibitors during homogenisation gives rise to an increase in the proportion of the 2.5S form of mouse NGF while the guinea pig material remains as the  $\beta$ -subunit. Of several possible explanations, two obvious ones are either that the guinea pig factor is less susceptible to enzymatic cleavage or that the appropriate proteases are present at lower levels in the prostate gland.

The levels of NGF in the guinea pig prostate gland have also been estimated using a two-site radioimmunoassay based on purified antibodies against mouse NGF<sup>18</sup>. The radioimmunoassay and biological assay results were in reasonable agreement,

around 35% of the levels expected from the biological assay being detected. The major cause of the discrepancy seems to be the use of antibodies raised against mouse NGF, only some of which recognise guinea pig NGF (see immunodiffusion results), and the capacity of the radioimmunoassay system is thereby substantially reduced. Details of the use of the radioimmunoassay to measure NGF from species other than the mouse will be published elsewhere.

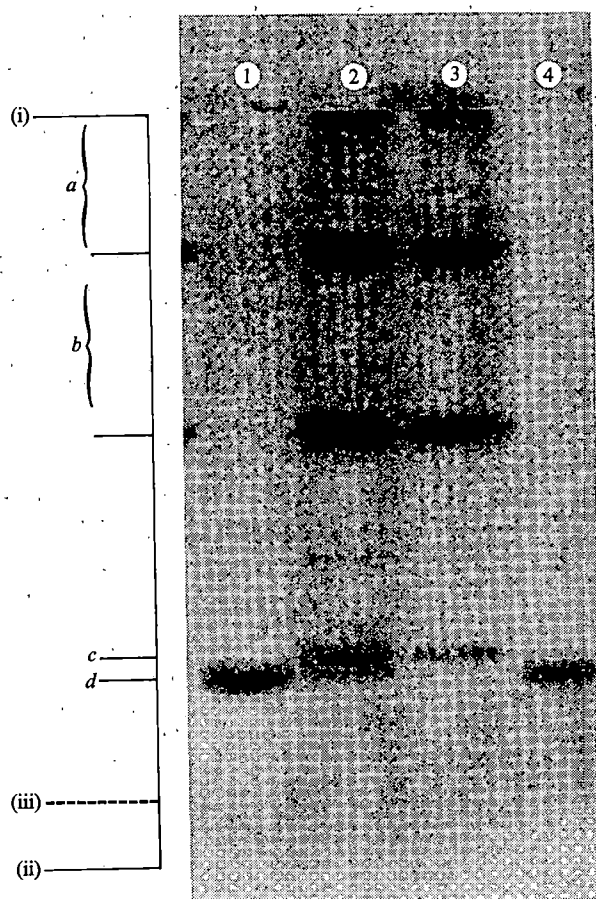
The observation that only a certain proportion of the antibodies to mouse NGF recognise guinea pig NGF contrasts with the similarity of the amount of antiserum required to block 1 BU of guinea pig NGF *in vitro* to the homologous titre. This could be due either to differences in the specific activities of the factors from the two sources or, possibly, to differences in the relationship between the antigenic sites and the sites associated with biological activity.

The significance of the presence in the accessory sex organs of agents with such potent effects on the sympathetic nervous system is not clear, but it is possible that the NGFs have a physiological role unconnected with, and in addition to, their effects on neuronal tissues. The levels of NGF in the mouse submaxillary gland show a well characterised developmental increase<sup>2</sup> and a marked sex difference<sup>19-21</sup>. A similar situation seems to apply in the reproductive system of the guinea pig. The high levels of NGF *in vivo* thus appear after the development of the sympathetic nervous system<sup>22,23</sup> and in the absence of any significant sexual dimorphism in the responsive neuronal tissues<sup>1,24,25</sup>. The actions of NGF on the sympathetic nervous system may require only low levels of the agents, which are attained (although not necessarily detected with the assays



**Fig. 1** Comparison of guinea pig and mouse NGFs by immunodiffusion. Wells 1,3,5: 20  $\mu$ l of an extract of guinea pig prostate gland, containing 240  $\mu$ g NGF per ml (by biological assay). The tissue extract was prepared in distilled water and the lyophilised material was further extracted in 50 mM ammonium acetate/acetic acid buffer pH 4.0, containing 1 mg ml<sup>-1</sup> bovine serum albumin, 200  $\mu$ g ml<sup>-1</sup> sodium azide, 50  $\mu$ g ml<sup>-1</sup> pancreatic trypsin inhibitor and 5 mM phenylmethanesulphonyl fluoride. The solution was centrifuged (22,000g, 30 min) to remove insoluble material, returned to pH 7.2 with NaOH, centrifuged again to clarify, and stored at  $-70^{\circ}\text{C}$ . This procedure removed many proteins from the crude extract but all the NGF was recovered, as judged by biological and radioimmunological assays. Wells 2,4,6: 20  $\mu$ l of a solution (in 50 mM Tris-HCl buffer pH 8.5) of purified mouse NGF<sup>18</sup>, at a concentration of 250  $\mu$ g NGF per ml. Well 7: 20  $\mu$ l of a solution (in 50 mM Tris-HCl buffer pH 8.5) of lyophilised sheep antiserum to mouse NGF<sup>18</sup> (60 mg ml<sup>-1</sup>). Immunodiffusion plates were made up of 0.4% agarose containing 0.1% Thimerosal. Plates were incubated at  $20^{\circ}\text{C}$  in a humidified atmosphere for 48 h, washed for 5 d in 150 mM NaCl solution to remove non-precipitated proteins, stained for 1–2 h in 0.25% Coomassie brilliant blue in 150 mM saline, destained for 3 d in 150 mM saline, and photographed. An identical result was obtained when wells 2, 4 and 6 contained an extract, prepared as described above, of mouse submaxillary gland, containing 160  $\mu$ g NGF per ml.





**Fig. 2** SDS-gel electrophoresis of immunoprecipitates of guinea pig and mouse NGFs. Two guinea pig prostate glands (7 ml) and two mouse submaxillary glands (5 ml) were homogenised with a Potter ground glass homogeniser in the volumes shown of 50 mM Tris-HCl buffer pH 7.4 containing 1% Triton X-100, 300  $\mu\text{g ml}^{-1}$  phenylmethanesulphonyl fluoride, and 20  $\mu\text{g ml}^{-1}$  pancreatic trypsin inhibitor. Homogenates were centrifuged at 22,000g (30 min) and stored at  $-50^\circ\text{C}$  until required; the supernatants were recentrifuged at 25,000g for 20 min after thawing and immediately before use. 100  $\mu\text{l}$  of guinea pig extract and 60  $\mu\text{l}$  mouse extract were incubated overnight at  $4^\circ\text{C}$  with 10  $\mu\text{l}$  Nonidet P40 detergent, 250 and 230  $\mu\text{l}$  respectively of a solution (in 50 mM Tris-HCl buffer pH 7.2, containing 150 mM NaCl) of purified sheep antibodies<sup>16</sup> to mouse 2.5S NGF (0.73 mg antibodies per ml) and Tris-NaCl buffer to a final volume of 400  $\mu\text{l}$ . Pilot experiments determined that these ratios of antigen to antibody were at the equivalence point. Immunoprecipitates were collected by centrifugation at 15,000g for 10 min and washed three times with 500  $\mu\text{l}$  Tris-NaCl buffer containing 2.5% (v/v) Nonidet P40. The immunoprecipitates were then dissolved in the sample buffer for electrophoresis (15% glycerol, 5% 2-mercaptoethanol, 2.25% SDS, 0.01% bromophenol blue and 0.0625 M Tris-HCl buffer pH 6.8) by heating at  $90^\circ\text{C}$  for 10 min. The solubilised immunoprecipitates were subjected to SDS-15% polyacrylamide gel electrophoresis according to the method of Laemmli<sup>29</sup>. The slab gel was performed in an apparatus similar to that described by Studier<sup>30</sup>. The slab gel was stained with 0.25% Coomassie brilliant blue in methanol acetic acid water (5:1:5 by volume) solution and destained in the same solution. (i), (ii), Top and bottom respectively of the SDS-polyacrylamide gel; (iii), bromophenol blue front. a, Immunoglobulin heavy chains; b, immunoglobulin light chains; c, position of the intact monomer of mouse  $\beta$  and 2.5S NGF, and of the guinea pig NGF; d, position of the smaller monomer from mouse 2.5S NGF. 1, Purified mouse NGF, 4  $\mu\text{g}$ . Both chains are entirely converted to the smaller chain, as found in 2.5S NGF, when NGF is purified according to ref. 18. 2, Mouse NGF immunoprecipitate. 3, Guinea pig NGF immunoprecipitate. 4, Purified mouse NGF 2  $\mu\text{g}$  (as above).

presently available) in developing (and female) animals. Another, possibly sex-linked, function is conceivable for these proteins, for which very high levels of NGF are required.

From a practical point of view, the high levels of NGF in the guinea pig prostate gland make this source an attractive alternative to the male mouse submaxillary glands. Interestingly, in view of the appearance of an epidermal growth factor (EGF) in the mouse submaxillary gland together with the NGF<sup>26-28</sup>, preliminary studies indicate that the guinea pig prostate gland could be a source of EGF of a potency comparable with or greater than the mouse submaxillary gland.

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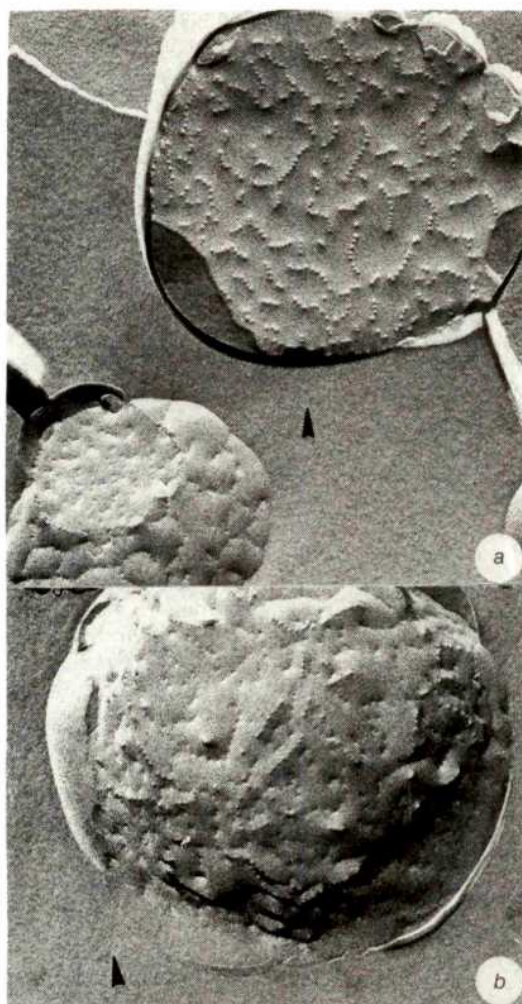
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## Lipidic intramembranous particles

THE nature of intramembranous particles has recently been discussed in relation to the mode of freeze fracturing<sup>1,2</sup>. It has been argued that particles that do not show complementarity (complementary pits on the opposite fracture face) are a reflection of protein penetrating the membrane. The protein





**Fig. 1** An equimolar mixture of egg-lecithin, isolated according to established procedures, and cardiolipin (bovine heart cardiolipin) with a  $\text{Ca}^{2+}$ /cardiolipin molar ratio of 0:1 is dissolved in ether and injected according to the method described by Deamer and Bangham<sup>8</sup> in a buffer containing 150 mM NaCl and 25 mM Tris, pH 7.5. The purity of the lipids was found to be at least 99% as evidenced by TLC on silica-gel G using the elution system chloroform/methanol/acidic acid (93:30:12). Shadowing direction is indicated by arrows.  $\times 52,000$ .

giving rise to an intramembraneous particle is plastically deformed during the fracturing procedure and is probably pulled out to one of the two fracture faces. Moreover we have argued that particles showing complementarity (pits) are possibly of lipidic origin. This latter hypothesis was mainly based on the determination of the nature of the intramembraneous particles of the outer membrane of *Escherichia coli*. These complementary particles were shown to be determined by lipopolysaccharide<sup>3,4</sup>. We show here that lipid can by itself form intramembraneous particles showing complementarity and that these particles may be inverted micelles of phospholipid sandwiched between lipid monolayers.

To test the hypothesis that lipid can give rise to intramembraneous particles with complementary impressions on the opposite fracture face, we have studied primarily lipids that can exist in non-bilayer phases. Good candidates are phosphatidylethanolamines from natural sources<sup>5</sup> and especially cardiolipin in the presence of  $\text{Ca}^{2+}$  (ref. 6) which can form the hexagonal phase II.

Recently we have shown that the transition of cardiolipin from the bilayer configuration to the hexagonal phase by addition of  $\text{Ca}^{2+}$  occurs by an intermediate phase of particles about 70 Å in diameter, as visualised by freeze fracturing<sup>7</sup>. This intermediate phase is characterised by the <sup>31</sup>P NMR spectrum

which indicates isotropic motional averaging. This phase, which exists at  $\text{Ca}^{2+}$ -cardiolipin ratios below 0.6, can be interpreted as inverted micelles, that is particles with hydrophilic cores and hydrophobic edges.

To see whether such inverted micelles can form intramembraneous particles we have prepared model membranes of an equimolar mixture of lecithin and cardiolipin at a low  $\text{Ca}^{2+}$  cardiolipin ratio as described in Fig. 1 legend.

Freeze fracturing shows fracture faces of bilayers organised predominantly in large unilamellar structures. Also some multilamellar structures are found. On the fracture faces of these bilayers particles of a rather uniform size are present. The mean diameter is 100 Å (s.d. 14 Å;  $N = 100$ ). The pits are about 30 Å smaller. Fracture faces bearing exclusively particles or exclusively pits are found (Fig. 1a) but also fracture faces with both particles and pits can be observed (Fig. 1b). If  $\text{Ca}^{2+}$  is removed by EGTA extraction, neither particles nor pits are observed—only smooth fracture faces. On the other hand excess  $\text{Ca}^{2+}$  results in a homogeneous conglomerate of globules about 100 Å in diameter. (These latter results will be published in detail elsewhere.)

The most important conclusion that can be drawn from these observations is that lipid by itself can form intramembraneous particles and that such particles show complementarity. These particles might arise from invaginations of the bilayer, but this is unlikely as one should expect a variation in the size of particles and consequently in the size of the pits, which is not the case. In our opinion these particles represent inverted micelles of phospholipid sandwiched between lipid monolayers.

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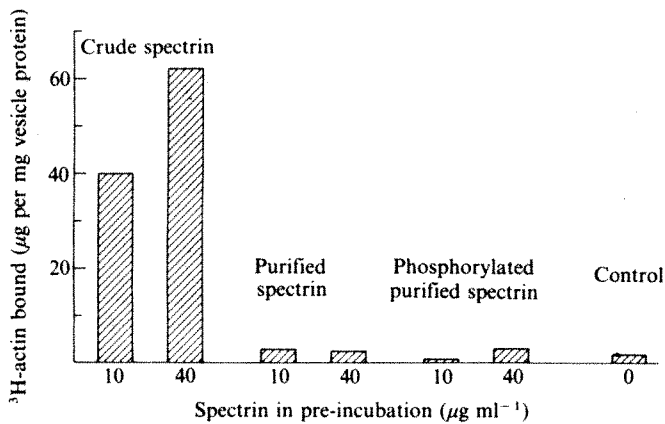
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## The role of spectrin in erythrocyte membrane-stimulated actin polymerisation

PROTEINS on the cytoplasmic surface of the erythrocyte membrane, including spectrin and actin, are postulated to comprise the red cell cytoskeleton<sup>1–3</sup>, but little is known about the role of actin or its association with the membrane. We have reported that monomeric (G) actin added to erythrocyte ghosts selectively associates with a component at the cytoplasmic surface of the membrane<sup>4,5</sup>. We now show that this component is unlikely to be spectrin and that actin binding occurs by stimulated actin polymerisation.

Large amounts of added G-actin bind to the cytoplasmic surface of unsealed ghosts, but little or none binds to sealed ghosts or spectrin-actin-depleted inside-out vesicles<sup>4,5</sup>. After spectrin-actin-depleted inside-out vesicles (referred to here





**Fig. 1** Actin binding to vesicles reconstituted by preincubation with spectrin. Unsealed, spectrin-actin-depleted inside-out vesicles were prepared from ghosts<sup>18</sup> of freshly drawn human erythrocytes by one wash in ice-cold 0.3 mM sodium phosphate buffer, pH 7.6, incubation for 40 min in 40 volumes of 0.3 mM sodium phosphate buffer at 37 °C and two washes in ice-cold 0.3 mM sodium phosphate buffer. Crude spectrin (primarily spectrin, actin and band 4.1) was prepared by extracting ghosts in 0.3 mM sodium phosphate buffer, 37 °C and phosphorylated by incubating 1 mg spectrin in 1 ml of 100 mM NaCl, 10 mM Tris pH 7.4, 5 mM MgCl<sub>2</sub>, 50 μM ATP containing 1 μCi of <sup>32</sup>P-ATP and 200 μg erythrocyte kinase<sup>19</sup> at 37 °C for 30 min. Saturation of <sup>32</sup>P incorporation occurred after ~20 min. (33 mol PO<sub>4</sub> per mol spectrin in this experiment.) After phosphorylation, the <sup>32</sup>P-spectrin was purified on a sucrose density gradient<sup>6</sup> (similar results were obtained using spectrin which was phosphorylated after gradient purification). Vesicles (0.3 mg protein per ml) were incubated for 1 h on ice with the indicated concentrations of spectrin in 20 mM KCl, 2 mM MgCl<sub>2</sub>, 5 mM sodium phosphate pH 7.6, and 0.75 mM β-mercaptoethanol, washed once and resuspended in this same medium to a protein concentration of 0.25 mg ml<sup>-1</sup>. Control experiments confirmed that the amounts of crude, pure or phosphorylated pure spectrin which bound to vesicles were comparable when they were added at the same concentration (~60 μg per mg vesicle protein and 90 μg per mg for added spectrin concentrations of 10 and 40 μg ml<sup>-1</sup> respectively). Preparation of [<sup>3</sup>H]G-actin from rabbit skeletal muscle and quantification of <sup>3</sup>H-actin binding as described previously<sup>5</sup>. Briefly, 40 μg ml<sup>-1</sup> [<sup>3</sup>H]G-actin was incubated in a volume of 0.8 ml with 0.25 mg ml<sup>-1</sup> membrane protein in 20 mM KCl, 5 mM sodium phosphate pH 6.5, 2 mM MgCl<sub>2</sub>, 1 mM ATP and 0.75 mM β-mercaptoethanol (actin-binding buffer) on ice for 1 h. Samples were layered on 0.2 ml of 20% w/v sucrose (made in actin-binding buffer) in 0.5 ml polyethylene microfuge tubes, centrifuged at 18,000 r.p.m. for 25 min in a Sorvall SS34 rotor and the tips, containing the membrane pellet, frozen in liquid N<sub>2</sub>, cut off, placed in 10 ml of scintillation fluid and counted for <sup>3</sup>H. No <sup>3</sup>H-actin sedimented through the sucrose in the absence of membranes in any of the conditions studied.

simply as vesicles) were reconstituted with crude spectrin (primarily spectrin, actin and band 4.1; for nomenclature see ref. 1), their ability to bind added G-actin became nearly equal to that of unsealed ghosts<sup>5</sup>. As the major component of crude spectrin is spectrin itself, we tested the ability of the purified protein to stimulate actin binding (Fig. 1). Crude spectrin was effective at stimulating actin binding to inside-out vesicles at low concentrations, but purified spectrin had little or no effect, even when added in concentrations sufficient to saturate the spectrin binding capacity (ref. 6, and our unpublished data) of the vesicles.

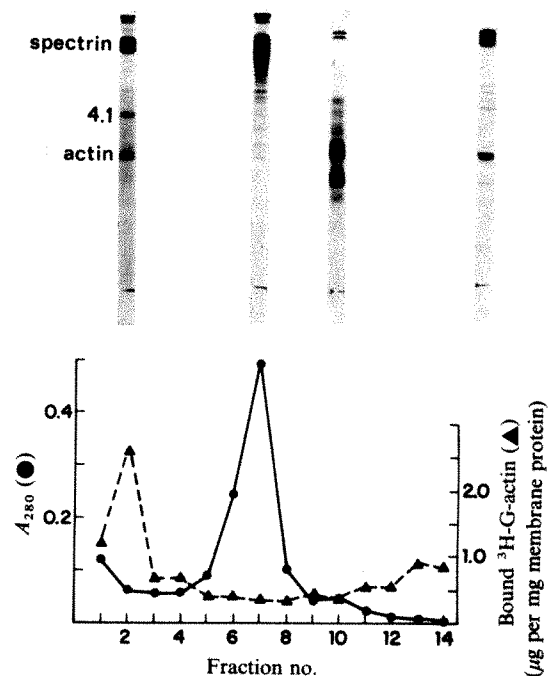
As the interaction of spectrin and actin has been reported to be sensitive to spectrin's state of phosphorylation<sup>7</sup>, we considered the possibility that the purified spectrin was inactive because it had become dephosphorylated. However, reconstitution of vesicles with phosphorylated spectrin did not stimulate actin binding (Fig. 1).

The ability of crude, but not purified spectrin to stimulate actin binding prompted us to explore the possibility that some component of crude spectrin other than spectrin itself was responsible for this effect. Crude spectrin was centrifuged on a 12-ml linear 5–20% sucrose gradient which was fractionated into 14 aliquots. Samples of vesicles were reconstituted with each of the gradient fractions and tested for their ability to bind added G-actin (Fig. 2). The only fraction capable of stimulating binding was found near the bottom of the gradient and contained spectrin, red-cell actin, and band 4.1. The ability of this fraction to stimulate binding was less than an equivalent amount of crude spectrin, indicating some loss of stimulatory activity during purification.

These results show that some component of crude spectrin other than spectrin itself, can stimulate actin binding to red-cell membranes. We propose that this stimulated binding occurs as a direct result of induced polymerisation of the added G-actin because: (1) actin binding depends on the presence of Mg. (2) Actin binding is non-saturable. (3) Actin binding can be prevented by DNase I and cytochalasin B<sup>5</sup>. (4) Actin binding is accompanied by terminal phosphate hydrolysis from the tightly bound ATP of the actin molecule<sup>8</sup>, a hallmark of actin polymer formation. In addition, we have shown that vesicles reconstituted with crude spectrin and subsequently incubated with G-actin become covered with actin filaments which emanate from their surface<sup>5</sup>. Decoration of these filaments with heavy meromyosin (Fig. 3) reveals that they all have the same polarity, arrow heads pointing towards the membrane, indicating that although polymerisation is nucleated by the membrane, growth proceeds at the free, distal end of the filament<sup>9</sup>. Thus, the polarity of the filaments is opposite to that seen *in situ* in other cell types<sup>10–13</sup>.

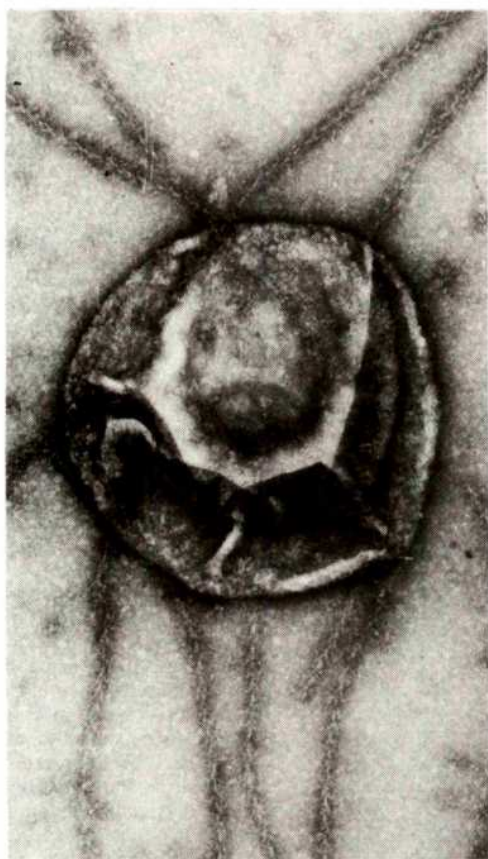
In line with our conclusion that crude spectrin stimulates actin binding to vesicles by polymerisation<sup>8,14</sup>, we have used a novel microassay for actin polymerisation<sup>8,14</sup> and found that crude spectrin can stimulate actin polymerisation in solution, whereas purified spectrin, whether phosphorylated or not, does not stimulate actin polymerisation. This suggests that there is a direct relationship between polymerisation-inducing capacity and stimulation of actin binding to membranes.

Our attempt to define the components of crude spectrin responsible for stimulating actin binding requires further exploration, as the gradient fraction capable of stimulating binding contains all three of the major components of crude spectrin (spectrin, red-cell actin and band 4.1). Their presence near the bottom of the gradient suggests that they may be associated in a supramolecular complex migrating with a sedimentation coefficient considerably greater than any of the pro-



**Fig. 2** Stimulatory effect of spectrin gradient fractions on actin binding by vesicles. Crude spectrin was centrifuged on a 5–20% sucrose gradient as described in Fig. 1. The gradient was fractionated, and 200-μl aliquots of each fraction were incubated in 1 ml of 20 mM KCl, 2 mM MgCl<sub>2</sub>, 5 mM sodium phosphate pH 7.6, 0.75 mM β-mercaptoethanol containing spectrin-actin-depleted inside-out vesicles (0.3 mg protein) for 1 h at 4 °C. Samples of vesicles reconstituted with each of the gradient fractions were washed once and resuspended in this same buffer to 0.25 mg protein per ml and tested for actin binding as described in Fig. 1. The absorbance of each gradient fraction (solid line) and actin bound by vesicles reconstituted with that fraction (dashed line) are plotted. 5% SDS polyacrylamide gels<sup>20</sup> of selected gradient fractions are shown above the corresponding fraction numbers, the gel to the far right being the crude spectrin.





**Fig. 3** Electron micrograph of negatively stained inside-out vesicles reconstituted with crude spectrin, incubated with G-actin and subsequently decorated with heavy meromyosin. Spectrin-actin-depleted inside-out vesicles were reconstituted with crude spectrin ( $40 \mu\text{g ml}^{-1}$ ) and incubated with  $40 \mu\text{g ml}^{-1}$  G-actin as described in Fig. 1. After incubation for 20 min with the actin, the vesicles were sedimented through 20% w/v sucrose to separate them from unbound actin and applied to a formvar-coated grid. The grids were rinsed three times with 0.6 M KCl, 10 mM Tris pH 7.6 followed by application of  $0.2 \text{ mg ml}^{-1}$  heavy meromyosin in the same medium. Grids were stained with 1% uranyl acetate.  $\times 230,000$ .

teins separately, but each of these proteins may be capable of forming multimeric associations with itself (spectrin is known to form tetramers and actin to form filaments). One or both of these factors may contribute to the formation of the active complex, but the presence of actin in the complex is of special significance because small quantities of purified F-actin, when reconstituted onto vesicles by the method used for crude spectrin or the gradient fractions, can stimulate the subsequent binding of added G-actin. Moreover, when purified F-actin is added to G-actin in solution at actin concentrations and solvent conditions identical to those used in our binding studies, rapid and extensive actin polymerisation takes place (data not shown). These findings suggest that crude spectrin and the active gradient fraction may contain F-actin, or actin aggregates, which are responsible for the polymerisation-related binding of added G-actin. This conclusion can be reconciled with the fact that unmodified red-cell ghosts and crude spectrin do not contain structures identifiable as F-actin by proposing that the actin on the membrane, and as extracted in crude spectrin, exists in small aggregates or proto-filaments consisting of three to five monomers. Such F-actin aggregates may be highly efficient in promoting filament formation in the presence of an excess of G-actin<sup>15</sup>, both in solution and when bound to red-cell membranes, and would allow red-cell actin to be bound and distributed in a homogeneous and apparently amorphous manner over the membrane surface.

Our hypothesis of membrane-bound actin aggregates can explain the membrane-induced actin polymerisation observed with ghosts and reconstituted inside-out vesicles, as well as crude spectrin-induced actin polymerisation in solution. Our

observation that purified spectrin has none of these activities, however, contradicts the results of Pinder *et al.*<sup>7</sup>, who measured viscosity changes and concluded that phosphorylated spectrin can induce actin polymerisation in solution. To reconcile our observations with those of Pinder *et al.*, we suggest that their spectrin, which was chromatographed on Sephadex G-200, contained red-cell actin aggregates which induced the polymerisation of added G-actin. Observations by other workers<sup>16,17</sup> provide clear evidence that spectrin prepared from the void volume fractions of this or other large-pore gels will be contaminated by small quantities of actin and other proteins. The effect of spectrin phosphorylation on actin viscosity might be to induce the cross-linking of adjacent filaments which had already been formed.

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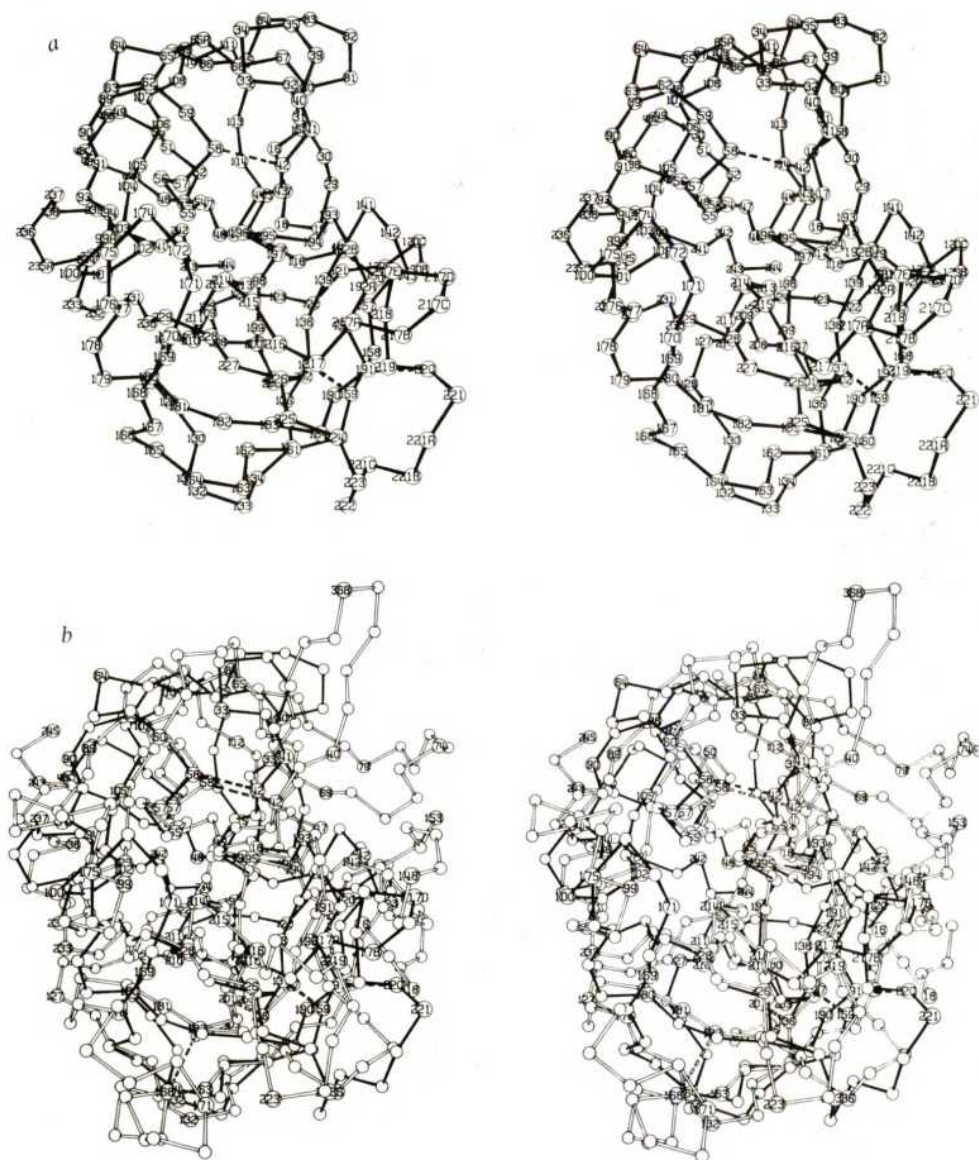
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## Comparison of the predicted model of $\alpha$ -lytic protease with the X-ray structure

THERE are several methods for the prediction of secondary structural features of protein molecules from the amino acid sequence<sup>1-5</sup>. Application of these methods to proteins of unknown tertiary structure has met with mixed results<sup>6,7</sup>, and it has been suggested that it is the protein structural type which will determine which predictive method will succeed<sup>8</sup>. There is also great interest in the *ab initio* prediction of tertiary structures of proteins<sup>9-11</sup>, but this has not yet been achieved. One possible method for predicting the tertiary structure of an enzyme is by the use of a known amino acid sequence and a previously determined tertiary structure of another related homologous isofunctional enzyme. This method was used by McLachlan and Shotton<sup>12</sup> in an attempt to fit the  $\alpha$ -lytic protease sequence into the polypeptide chain folding of elastase<sup>13</sup> and chymotrypsin<sup>14</sup>. It has also been used to predict the tertiary structure of tropo-nin C (ref. 15). Now that the structure of  $\alpha$ -lytic protease is known<sup>16</sup> to a resolution of 2.8 Å, we can assess the accuracy of





**Fig. 1** *a*, A stereo drawing of the  $\alpha$ -carbon atom backbone of  $\alpha$ -lytic protease, with each  $\alpha$ -carbon atom position numbered according to that of chymotrypsinogen A as described in detail in ref. 20. The active site region is located in the central portion of this drawing, where  $\alpha$ -carbon atom positions of the catalytic quartet of Ser 214, Asp 102, His 57 and Ser 192 are evident. The three disulphide bridges 42–58, 137–159 and 191–220 present in this molecule are denoted by dashed virtual bonds. *b*, The  $\alpha$ -carbon atom drawing of  $\alpha$ -lytic protease (solid virtual bonds) is superimposed on that of elastase (open virtual bonds) in a manner designed to maximise topological equivalences<sup>24</sup>. Every fifth  $\alpha$ -carbon atom position of each enzyme is numbered; additional numbering is also present for residues discussed in the text. See Fig. 1 of ref. 12 for more details of the model proposed.

such predictions. The tertiary structures of two related bacterial serine proteases, SGPA and SGPB and their structural relationship to the pancreatic enzymes have been published previously<sup>17–19</sup>. Many of the conclusions drawn from those studies are also applicable to the present discussion on  $\alpha$ -lytic protease and need not be repeated. Rather, we shall consider the basic premise of sequence homology in phylogenetically distant proteins being used to deduce tertiary structures. A recent realignment<sup>20</sup> of the amino acid sequences of Gly-Asp-Ser-Gly-Gly proteases, which was based on the known topological equivalences of  $\alpha$ -carbon atoms, indicates an overall sequence identity of only 18% between  $\alpha$ -lytic protease and elastase. We show here that this low value is independent of the environment of the topologically equivalent polypeptide chains (whether the residues are internal or external) and that the sequence identity is associated with only those residues which are in the immediate vicinity of the active site quartet, Asp 102, His 57, Ser 195 and Ser 214.

The complete amino acid sequence determination of this bacterial serine protease from *Myxobacter* 495 revealed short stretches of sequence which were highly homologous to presumably corresponding regions in the sequences of the mammalian pancreatic enzymes—chymotrypsin, trypsin and elastase. This homology and the similar catalytic behaviour towards peptide substrates<sup>22</sup> suggested that  $\alpha$ -lytic protease might have a tertiary structure similar to that of the mammalian enzymes despite the fact that it has a much smaller molecular

weight than porcine elastase (19,900 compared to 25,900). In regions of the polypeptide chain of  $\alpha$ -lytic protease which do not contain residues of the active site, the sequence homology with the pancreatic enzymes is so low that serious sequence misalignments have resulted<sup>12,21</sup> and persisted even with the knowledge of the tertiary structure of SGPB<sup>17</sup>. In spite of the lack of sequence homology, there is considerable tertiary structural equivalence which is far more extensive and presumably more conserved than the amino acid sequence identities would suggest.

The polypeptide chain folding of  $\alpha$ -lytic protease (represented by  $\alpha$ -carbon atom positions only) is illustrated in Fig. 1*a*. Figure 1*b* shows the  $\alpha$ -carbon atoms of  $\alpha$ -lytic protease superimposed on the  $\alpha$ -carbon atom backbone drawing of elastase<sup>23</sup> (coordinates for elastase are from the Brookhaven Protein Data Bank). The superimposition of these two enzyme structures was achieved using a computer program written by W. Bennett of Yale University and is based on the methods described by Rossmann and Argos<sup>24</sup>. Examination of Fig. 1*b* shows that there is indeed topological equivalence of  $\alpha$ -carbon atoms between  $\alpha$ -lytic protease and elastase. However, this tertiary structural homology<sup>20</sup> (55% of the  $\alpha$ -carbon atoms of  $\alpha$ -lytic protease (108 residues) have a topologically equivalent  $\alpha$ -carbon atom in elastase within an r.m.s. deviation of 2.08 Å) is limited to the central core, and in particular to those stretches of polypeptide chain which support or contain residues directly involved with substrate binding or the catalytic event.

An alternative representation of the topological equivalence between  $\alpha$ -lytic protease and elastase is given in Table 1 which summarises those 76 residues in both domains of these enzymes (domain I, residues 16–120; domain II, 121–245) which have the highest degree of topological equivalence. It is clear from Table 1 that the number of topologically equivalent residues in domain II is more than double that in domain I. This is also true if the topological comparisons are done treating each domain independently. There are only three strands of polypeptide chain which superimpose well in domain I and these strands support the two catalytically important residues His 57 and Asp 102. The apparently high level of amino acid identity (36%) is the result of the small absolute number of topologically equivalent residues (22) in domain I.

Domain II has 54 topologically equivalent  $\alpha$ -carbon atoms in six strands of antiparallel  $\beta$ -pleated sheet. The degree of sequence identity between  $\alpha$ -lytic protease and elastase is relatively low (22%). There is only one strand (that supporting Ser 195) which has extensive sequence identity, as shown in Table 1. The lack of sequence homology between the first three topo-

logically equivalent strands of domain II is not sufficient to predict a correct model successfully. Indeed, there is only one residue that is structurally required out of the 25 residues comprising these three strands. Gly 140 is structurally invariant<sup>20</sup> and is required as such; any side chain at this position would sterically interfere with the side chain of Asp 194, a residue essential to determining the proper catalytically active conformation of serine protease binding sites<sup>25,26</sup>. The three strands of topologically equivalent residues in domain I of  $\alpha$ -lytic protease were correctly predicted from the elastase fold<sup>12</sup> as were strands 4 and 6 domain II. However, the remaining four strands, although topologically equivalent in the two enzymes, had so little sequence homology that the correct register was not discerned. Clearly, what is conserved throughout the evolution of these important digestive molecules is the three-dimensional structure and not the amino acid sequence, except in the immediate vicinity of the catalytic quartet.

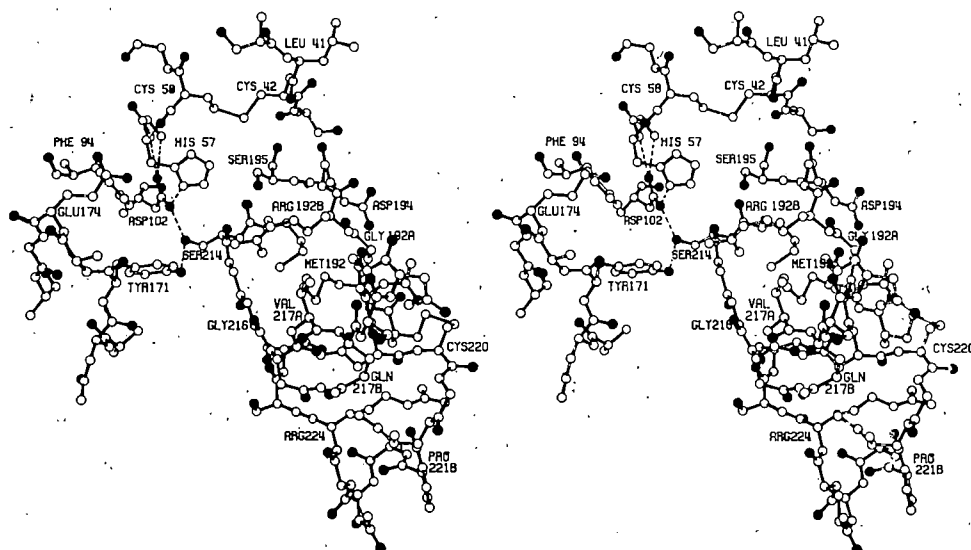
The  $\alpha$ -lytic protease and pancreatic elastase have a similar substrate specificity<sup>22,27</sup>. The steric interactions whereby these two structurally related enzymes achieve this specificity for small

Table 1 Topological equivalence and sequence homology of  $\alpha$ -lytic protease and elastase

[A] Domain I (residues 16–120)															
$\alpha$ -LP Elastase	$\Delta$							41	42	43	44				
								L T	C C	S G	V G				
								0.93	1.37	1.21	0.94				
$\alpha$ -LP Elastase	$\Delta$		51	52	53	54	55	56	57	58	59				
			G W	F V	V M	T T	A A	G A	H H	C C	G V				
			1.03	0.96	0.49	0.39	0.33	0.46	1.04	1.54	0.58				
$\alpha$ -LP Elastase	$\Delta$	109	108	107	106	105	104	103	102	101					
		T A	L L	S R	V L	W L	A A	R I	D D	N Y					
		1.72	0.89	1.21	0.81	0.43	0.83	1.21	0.57	2.17					
[B] Domain II (residues 121–245)															
$\alpha$ -LP Elastase	$\Delta$	131	132	133	134	135	136	137	138	139	140	141	142		
		A A	V N	G N	A S	A P	V C	C Y	R I	S T	G G	R W	T G		
		1.32	1.87	1.57	1.53	1.69	0.50	1.20	0.95	1.11	0.70	1.54	1.82		
$\alpha$ -LP Elastase	$\Delta$			163	162	161	160	159	158	157	156				
				T V	I T	T P	G L	C Y	Q A	Y Q	G Q				
				1.38	0.42	0.76	2.68	1.08	1.00	1.35	1.20				
$\alpha$ -LP Elastase	$\Delta$	180	181	182	183	184									
		L M	T V	Q C	G A	N G									
		1.32	1.33	1.01	1.49	1.21									
$\alpha$ -LP Elastase	$\Delta$	191	192	192	192	193	194	195	196	197	198	199	200		
		C S	M G	G C	R Q	G G	D D	S S	G G	G G	S P	W L	I H		
		1.41	0.99	1.75	1.06	0.87	0.29	0.25	0.82	1.22	0.45	0.26	0.79		
$\alpha$ -LP Elastase	$\Delta$					216	215	214	213	212	211	210	209	208	207
						G V	G F	S S	M T	V V	G G	Q H	A V	Q A	G Y
						0.97	0.94	0.73	0.60	1.14	1.33	0.93	1.63	1.52	1.09
$\alpha$ -LP Elastase	$\Delta$					225	226	227	228	229	230	231			
						S P	S T	L V	F F	E T	R R	L V			
						1.82	0.83	0.73	0.37	0.50	1.72	1.18			

Those residues enclosed in boxes are chemically identical in  $\alpha$ -lytic protease and elastase. The amino acid sequences are expressed in the 1 letter code<sup>30</sup> and have been taken from ref. 20.  $\Delta$  is the r.m.s. deviation of the  $\alpha$ -carbon positions after optimisation and is expressed in Å units. The average r.m.s. deviation for domain I is 0.96 Å; for domain II, 1.12 Å. Those residues which are buried (internal) have a line drawn over the residue number. The strands are drawn diagrammatically to represent the antiparallel  $\beta$  hydrogen bonding seen in Fig. 1.





**Fig. 2** Stereo drawing of the active site of  $\alpha$ -lytic protease. The polypeptide main chain bonding is shown with solid black bonds. All oxygen atoms present are distinguished by solid black circles. Hydrogen bonds involving active site residues are illustrated as dashed lines. The side chains of Met 192, Met 213 and Val 217A occlude the primary binding pocket. The conformational lability of Arg 192B would not contribute to such a blockage of the substrate binding site.

side chains (Ala, Val, Ser) are quite different and were not predicted from model building<sup>12</sup>. The active site and substrate binding region of  $\alpha$ -lytic protease is shown in Fig. 2. The primary specificity site of the pancreatic-like serine enzymes involves the residues from 191–195 and 214–220. Elastase achieves its specificity because of the presence of Val 216 and Thr 226 (ref. 13), both side chains of which block the entrance to a hydrophobic cavity that exists in  $\alpha$ -chymotrypsin<sup>4</sup>; the topologically equivalent residues in  $\alpha$ -chymotrypsin are Gly 216 and Gly 226 (ref. 20). Residue 216 is also a glycine in  $\alpha$ -lytic protease and therefore the structural reason for its specificity must differ from that of elastase and from that proposed<sup>12</sup>.

Two important insertions in the sequence of  $\alpha$ -lytic protease as compared with that of elastase occur in the active site region<sup>20</sup>. The first of these insertions is a dipeptide at position 192. As a consequence, the side chain of Met 192 occludes the specificity pocket of  $\alpha$ -lytic protease, whereas the side chain of Gln 192 in elastase is directed away from this binding pocket. The second insertion is a pentapeptide at position 217. The side chain of Val 217A of this insertion is also directed into the specificity pocket (see Fig. 2). Consequently, the primary binding site of  $\alpha$ -lytic protease has room for substrate residues with side chains only as large as that of a valine residue<sup>16</sup>. In the region of residues 214–220, McLachlan and Shotton had misaligned (by four residues) the sequence of  $\alpha$ -lytic protease with that of elastase and had placed a glutamine residue at position 216 and concluded that this residue in  $\alpha$ -lytic protease would diminish the size of the primary specificity site, as does Val 216 in elastase. However, this glutamine residue is really residue 217B in the sequence and is located on the surface of the enzyme with its side chain pointing away from the active site (Fig. 2). Moreover, that misalignment had placed an asparagine residue at position 214 whereas a topologically equivalent serine residue has been found at this site in all of the serine protease structures that have been reported to date<sup>16–19,23,26,28</sup> (including the evolutionarily unrelated subtilisin<sup>29</sup>).

The extensive degree of structural similarity of these two serine proteases, in spite of the lack of sequence identity of topologically equivalent residues, makes it necessary to evaluate the expectations of a structural prediction. Our comparison of the 2.8-Å structure of  $\alpha$ -lytic protease with the 2.5-Å structure of elastase shows that the molecules are very similar in the regions near those residues important for catalysis. However, it does not seem possible to rely on primary structural homology (or lack thereof) to predict correctly and accurately an unknown structure from that of an isofunctional protein molecule of known tertiary structure. Resort to secondary structure predicting rules<sup>1–5</sup> would not necessarily offer much help, for in the stretch 131–138 of  $\alpha$ -lytic protease an  $\alpha$ -helical conformation is

incorrectly predicted whereas the corresponding region of elastase is indifferent to either  $\alpha$  or  $\beta$  structure<sup>1</sup>. If the results of protein structure prediction are to be used only to detect the possibility of conformational similarity and thus evolutionary homology, then such techniques can be useful<sup>12,15</sup>. However, if the interest is in the structural detail of substrate binding sites, or, in the conformation of the catalytically important residues, then the present methods of predicting structure are not satisfactory.

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# matters arising

## Human activity and the erosion of soils on chalk

IN their letter<sup>1</sup> relating to beech bark disease and lynchets, Lonsdale *et al.* refer to the probability of an acid non-calcareous soil overlying the chalk on their site. This type of soil seems to be very widespread in the west Sussex-east Hampshire region, more so than the chalky soil which is generally supposed to be the characteristic one, and is by no means always underlain by Pleistocene or earlier deposits. In fact, on shallow slopes, a highly calcareous soil seems to occur only where human disturbance has facilitated erosion; this usually means in ploughed fields or on the negative aspects of lynchets such as Lonsdale *et al.* have observed. Steep slopes, including the chalk scarp, are, on the other hand, often calcareous. The wood in which Lonsdale *et al.* are working does indeed seem to contain the non-calcareous soil, although not in its most acid form; samples taken from it<sup>2</sup> in 1970, outside the lynchet area, were mostly found on analysis to have a non-calcareous, non-iron, mineral content of ~70%. They are significantly less calcareous than samples from the neighbouring ploughed fields, and this difference has been found in several such areas of old woodland cover and arable, even though the two resemble each other in topographic and other respects.

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## Aerosol anomalies preceding earthquakes

IN his article on aerosol anomalies preceding earthquakes Tributsch<sup>1</sup> suggests that there is emission of positive ions

before an earthquake occurs and that these ions are responsible for the many reports of animal disturbance before the major event itself. We have been investigating electrical phenomena associated with stress and failure in rocks and ceramics and some of our data may be applicable to Tributsch's hypothesis.

For testing, we normally attach connections to the rock via conductive epoxy or hammered-in phonograph needles. These points are connected to one or more electrometers before the specimen is placed in compression or bending. As the load is applied we see a series of pulsed currents that we associate with piezoelectric effects. As the load is increased there is a steady state current of the order of  $10^{-10}$  A that we associate with the stress induced migration of ions<sup>2</sup> or the inception of a continuous series of internal microfractures. In any case the current pattern and areas of highest current can be used to predict areas of high residual stress and the location of ultimate failure.

As the specimen approaches failure we observe the emission of both electrons and positive ions with energies up to 15 eV at current levels of  $10^{-11}$  A. If the experiment is done in a darkened room there are flashes of light as the material begins to fail. These flashes are not observed in a helium environment but are intensified in a water vapour or pure oxygen environment; we suggest that the electrons and ions are exciting water or oxygen molecules that decay by photon emission. This electron and ion emission may well be responsible for the effects discussed by Tributsch as well as the earthquake light phenomena reported by Derr<sup>3</sup>.

A full report on this work will be given at the Fourth International Congress on Rock Mechanics in September at Montreux.

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## Historical climatology

WE should like to join Professor H. H. Lamb in clarifying a point in our article on historical climatology<sup>1</sup>. We stated that the correlation between Lamb's English winter severity index values for the decades between AD 1100 and 1400 and Alexandre's decadal indices for winters in Belgium was very low. We attributed this largely to the fact that Alexandre used thoroughly criticised and carefully evaluated historical data, whereas the sources available to Lamb when he pioneered the method were compilations of variable but generally poor quality.

It is necessary to add that in using the indices to reconstruct temperatures, Lamb himself expressly avoided undue emphasis on the decadal values as such, instead relying on half-century mean values derived from them<sup>2</sup>. These smoothed values correlate much better with Alexandre's series. It is important to emphasise Professor Lamb's caution in this respect. Indeed, it was because his caveat evidently required reiteration that we originally chose to highlight the problems inherent in the decadal values.

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## The impossibility of comminuting small particles by compression

KENDALL<sup>1</sup> has given a simple explanation of the impossibility of comminuting and crushing a brittle body by cracking when its size is smaller than a certain critical value. This size effect was demonstrated by applying the classical Griffith<sup>2</sup>

energy criterion to a brittle body of idealised geometry. However, it has been shown recently<sup>3</sup> that the Kendall theory could be improved, and brought into complete agreement with the Griffith theory, if allowance were made for the restraint to the free lateral movement inevitably experienced by the brittle body during compression. Assuming the lateral restraining force to be a small fraction,  $\alpha$ , of the axial compression,  $F$ , and following exactly Kendall's<sup>1</sup> procedure and notation, the cracking force is

$$F = \frac{1}{(1 - w/d + 8\alpha c/d)} \left( \frac{2ERd}{3} \right)^{1/2} \quad (1)$$

where  $c$  is the crack length.

The force at the transition from cracking of the brittle body to its gross yielding under the platen is given by the solution to the quadratic

$$\frac{1}{Yd} \left( \frac{F}{b} \right)^2 - \frac{F}{b} (1 + 8\alpha c/d) + \left( \frac{2ERd}{3} \right)^{1/2} = 0 \quad (2)$$

In particular, if the particle size is reduced to a critical value

$$d_{crit} = \frac{32ER}{3Y^2(1 + 8\alpha c/d)^4} \quad (3)$$

cracking becomes impossible. Equations (1)–(3) differ from their counterparts in Kendall's paper by the presence of the very crucial term  $8\alpha c/d$ .

Based on my experimental data<sup>3</sup> and that of Kendall<sup>4</sup>, I have argued that the restraining force would be very small especially if the size of the body is reduced. In fact, a value of  $\alpha$  between 1/100 and 1/200 seemed most appropriate.

For the model material (polystyrene) tested by Kendall<sup>1</sup> the  $c/d$  ratio in equation (3) for all samples was 1.25. Equation (3) suggests that for  $\alpha = 1/200$  the transition from brittle to ductile behaviour of polystyrene could be expected at a size of 3.69 mm as opposed to 4.48 mm predicted by Kendall's theory. The experimentally observed value was 3.6 mm (ref. 1). Expression (3) clearly is in better agreement with experimental data. Likewise, equation (3) predicts a crushing limit of around 0.85  $\mu$ m for calcium carbonate which is also in close agreement with the average particle size of 0.8  $\mu$ m to which calcium carbonate is reduced after prolonged milling.<sup>1</sup>

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## Agonist regulation of $\alpha$ -adrenergic receptor numbers

THERE have recently been several reports which indicate that agonist-induced desensitisation of cells might be explained, at least in part, by reductions in the number of receptors exposed at the surface of target cells; the evidence has been particularly clear for  $\beta$ -adrenergic receptors. Thus, it is not surprising that studies have been designed to see whether agonist-induced desensitisation of cells to  $\alpha$ -adrenergic stimulation might be explained in a similar way. Two reports have now described fairly rapid agonist-induced decreases in  $\alpha$ -receptor number<sup>1,2</sup>, and another described a modest rise in  $\alpha$ -receptor number in animals treated for several weeks with reserpine to induce supersensitivity<sup>3</sup>.

In their report, Cooper *et al.*<sup>2</sup> seemed to consider that the observed reduction in receptor number provided an adequate explanation for the desensitising effects of agonists; no other mechanisms were apparently considered. However, this position is untenable, as their main observation was that a 50% decrease in receptor number in adrenaline-treated platelets (assessed by dihydroergocryptine binding) was accompanied by an essentially complete loss of two cellular responses to the same agonist (aggregation and 5-hydroxytryptamine secretion).

Surely, this must mean that only a small proportion of the desensitising effect of agonist treatment can have been mediated through the change in receptor number? Most of this desensitising effect was presumably a result of some event which was brought about by receptor activation but which did not involve any changes in the number of exposed receptors. This is hardly surprising, as there is much evidence which indicates that in other tissues, especially smooth muscles, changes in receptor numbers are not likely to be the main mechanism whereby increased or decreased exposure to agonists brings about changes in cell sensitivity to those ligands which act at  $Ca^{2+}$ -mobilising receptors<sup>4–6</sup> (of which the  $\alpha$ -adrenergic receptor is one type<sup>7</sup>). These alternative mechanisms for changes in sensitivity may not be as conceptually simple nor as well understood as the changes in receptor number, but they cannot be ignored when the available data are incompatible with an explanation based solely on changes in receptor number.

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ALEXANDER AND HANDIN REPLY—Michell is correct, of course, in stating that agonist-induced desensitisation of physiological responses might not be explainable on the basis of a 40–50% decrease in receptor number, as determined by ligand binding with <sup>3</sup>H-dihydroergocryptine (DHEC), an  $\alpha$ -adrenergic antagonist. It was not our intention to imply that this was the sole explanation for adrenaline-induced desensitisation in the platelet. However, we do not agree "that only a small proportion of the desensitising effect of agonist treatment can have been mediated through the change in receptor number". Since submission of our paper, there has been a report on the desensitisation of  $\beta$ -adrenergic receptors showing that the percentage of receptors identified by agonist binding is decreased to an extent considerably greater than the 35% decrease in receptor number determined by antagonist binding. These data suggest that agonists and antagonists bind different forms of the  $\beta$ -adrenergic receptor<sup>1</sup>. It has been reported that  $\alpha$ -adrenergic agonists and antagonists bind to distinct states of the  $\alpha$ -adrenergic receptor and that <sup>3</sup>H-DHEC may label both the agonist and antagonist state<sup>2–5</sup>. If most of the decrease in <sup>3</sup>H-DHEC binding in platelets preincubated with (–)-adrenaline represents changes in the putative agonist state, then the observed decrease in receptor number may be sufficient to explain most of the  $\alpha$ -adrenergic agonist-induced desensitisation in platelets. This possibility requires further investigation using both agonist and antagonist binding assays.

The references on denervation supersensitivity may not be relevant to our work on desensitisation. In fact, it has recently been reported that  $\alpha$ -adrenergic receptors in rat cerebral cortex increase after chronic reserpine treatment, suggesting that an increase in receptor number may contribute to post-junctional supersensitivity<sup>6</sup>.

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# reviews

## Concepts of colour

J. D. Mollon

*Color Vision: An Historical Introduction.* By G. S. Wasserman. Pp. 224. (Wiley: New York and Chichester, UK, 1978.) £13.40.

THOSE who study colour vision are very aware of the history of their field. There are two reasons for this. First, the topic is one that has recommended itself to the strongest intellects: Newton, Thomas Young, Maxwell and Helmholtz have left writings that set models of experimental elegance and theoretical clarity for the modern researcher. Second, the advancement of the field has depended on an instructive sequence of conceptual insights, which must be recapitulated by each fresh student. It is therefore very appropriate that Professor Wasserman should adopt an historical organisation in his intermediate-level textbook on colour vision.

As actual history, Wasserman's account is patchy and largely limited to expositions of the main printed sources. His summary of the relevant parts of Newton's *Opticks* is accurate and very clear; but his treatment of other early material is unashamedly based on secondary sources and he entirely omits mention of the trichromatists of the first half of the eighteenth century. His account of the contribution of Maxwell and Helmholtz is excellent, but then he spoils himself by recommending that the three-receptor theory of colour vision, which is widely referred to as the Young-Helmholtz theory (and which is almost certainly correct), should instead be eponymously linked with Newton and Maxwell. That Maxwell should enjoy more credit is fair; but to associate the theory with Newton is to confound the whole history of the field. The most fundamental fact about colour vision is that of trichromacy, the fact that only three variables are needed in a colour-matching experiment; and it is sometimes said that trichromacy is implicit in Newton's colour circle and centre-of-gravity rule. But (as Wasserman himself very clearly explains) by mixing three primary colours that lie

on the circumference of Newton's circle, one can reach only that part of colour space enclosed by a triangle inscribed within the circle. Newton lacked the concept of imaginary primaries lying outside the colour circle; and he lacked that concept because he lacked in turn the idea of narrowly tuned transducers, supposing only that "... the Rays of Light in falling upon the bottom of the Eye excite vibrations in the *Tunica Retina*" (Qu. 12) and that these vibrations are "propagated through the solid, pellucid and uniform Capillamenta of the optic nerves into the place of sensation" (Qu. 23). The "several sorts of Rays" merely "make Vibrations of several bignesses, which according to their bigness excite Sensations of several Colours" (Qu. 13). As Newton understood neither the fact of trichromacy nor the trichromatic theory that was later introduced to explain it, I urge colleagues not to adopt Wasserman's suggestion that we should speak of the Newton-Maxwell theory.

The topics covered by Wasserman include colorimetry, colour blindness, component and opponent theories, zone theories, photometry, physiology. There are detailed discussions of the theories of Hurvich and Jameson and of Guth; of Land's experiments on colour contrast; of heterochromatic photometry; and of the early microspectrophotometric records. Wasserman has taken great pains to elucidate conceptual difficulties for students and this trouble is repaid by a very lucid text. I very much liked his discussion of imaginary primaries and his account of non-additivities of heterochromatic lights. There is a very helpful passage relating Granit's early reports of retinal 'dominators' and 'modulators' to the later electrophysiology.

But the book has two weaknesses. First, the coverage is unbalanced (the British come off especially badly). Rushton's reflection densitometry is dismissed as valueless in a sentence. Stiles' increment-threshold method passes entirely unmentioned, although the spectral sensitivities so derived are

close to linear transformations of the colour-matching functions and (from this evidence and from microspectrophotometry) now seem likely to represent the sensitivities of the cones themselves. (Indeed, although the book is illustrated with a great number of spectral sensitivities, amazingly there is nowhere shown a set that resembles the ones likely to be correct.) Nothing is said of one of the most interesting developments of the last decade, Zeki's demonstration of areas in the prestriate cortex that seem to be specialised for the analysis of colour.

Second, Wasserman has some heresies of his own to press upon the unsuspecting student. Thus the chapter on colour blindness is quite out-of-date and misleading and the author perpetuates one of the most notorious conceptual errors of the field, that of supposing that anomalous trichromacy can be explained by giving abnormal weightings to the signals from the three cones of normal vision. There is a lot of dubious talk about two-peaked photo-pigment sensitivities in invertebrates and in vertebrates, without any mention to the student that invertebrate pigments may be bistable (the two states corresponding superficially to a rhodopsin and a metarhodopsin) or that the evidence for visually significant short-wave peaks in vertebrate pigments is very slight. The recent microspectrophotometric measurements of goldfish receptors shown on p189 do indeed exhibit secondary *cis*-peaks, but the goldfish pigments are based on vitamin A<sub>2</sub> and Wasserman does not point out that the *cis*-peak of the long-wave human pigment (P565) would lie well below 400 nm, at wavelengths completely absorbed by the lens of the eye.

In summary, Wasserman's book is good in parts. Should this Curate's Egg be given to students? Only if accompanied by many, well placed pinches of salt. □

J. D. Mollon is Lecturer in Experimental Psychology at the University of Cambridge, UK.

## Biological properties of immunoglobulins

*Comprehensive Immunology*. Volume 5: Immunoglobulins. Edited by R. A. Good and G. W. Litman. Pp. 381. (Plenum Medical: New York and London, 1978.) \$18.58.

THIS is a multi-authored book containing 16 different articles. The style of each is governed more by the authors themselves than by any unifying editorial criterion. The length of the chapters is also very variable and bears no relationship to their general importance. Whether this is by chance or design, it turns out to be fortunate because, in general, the most extensively, and to my mind the better, reviewed subjects have also suffered the least from the unavoidable delay in publication of books of this nature.

The major emphasis is on the more classical immunochemistry problems concerned with the nature of the antibody-antigen interaction. Crystallographic, physicochemical, kinetic and thermodynamic data bring us a comprehensive picture of the general properties of the antibody-combining sites and, more importantly, of the outstanding and unresolved aspects of the problem. For those of us trying to understand the peculiarities of monoclonal antibodies in serological reactions, some of the chapters (particularly that by Karush on "The Affinity of Antibody: Range, Variability, and the Role of Multivalence") are very relevant. Yes, Cathou was right in saying, "Immunochemistry does not appear to be in any danger of an early death" (p76).

The first six chapters, which also include discussions on the structural basis of the effector functions of antibodies and other biological properties, take up well over half of the book. The other portion doesn't attempt a comprehensive coverage of other aspects of immunoglobulins, but a quick glance at the table of contents may give a misleading impression of the real subject matter. For instance, the short discussion by Ohno on the significance of gene duplication in Ig evolution is intended for the initiated and not to give a picture of the complexities of the contraction and expansion processes in the evolution of immunoglobulin gene pools. On the other hand, I found a considerable amount of information on subjects that are seldom reviewed: for example, on immunoglobulin-like molecules and possible functional ancestors of antibodies in lower species (chapter 8) and on the relative expression of  $V_H$  subgroups in

normal and abnormal immunoglobulins in man (chapter 13).

Chapter 10 reviews the structure of atypical immunoglobulins and their implications in terms of genetic control mechanisms. It is a great pity that Frangione did not have an extra year to write this chapter, as his pioneering ideas about a separate 'gene' for the hinge region could have crystallised with the discoveries of 'gaps' in the DNA structure. The book closes with a crisp "Overview on membrane immunoglobulins": the outstanding problems, the relevant facts and the prevailing opinions at the time of publication. Pernis has obviously made a great effort to try to include last-minute references and, in the rush, Berget *et al.* (1977) did not make it to the reference list.

A number of errors have crept into

the book here and there, some of which seem worthy of mention. For instance, Wang (chapter 9) refers to Huang as "Huong", and in chapter 10 section 4.1, called "Internal Deletions", the word "deleted" at the end of the first sentence is missing (deleted?).

According to the flap of my copy, the book was intended to "prvide" (*sic*) a foundation for the subsequent interpretation of the biological properties of immunoglobulins. It is difficult to say if it does *that*, but I think it is a serious contribution to the increasingly effective communication between experts talking such different languages.

César Milstein

César Milstein is Head of the Protein Chemistry Subdivision, Medical Research Council Laboratory of Molecular Biology, Cambridge, UK.

## Field theory

*Relativistic Quantum Fields*. By C. Nash. Pp.223. (Academic: London, New York and San Francisco, 1978.) £15; \$31.

IN the past five years we have witnessed an astonishing renaissance of field theory in elementary particle physics. On the one hand the Weinberg-Salam unified theory of weak and electromagnetic interactions has had considerable success in fitting a large variety of neutrino neutral current data; on the other hand quantum chromodynamics (QCD) predicted and explains the scaling violations observed in deep inelastic neutrino scattering. As a result it is now the conventional wisdom that these theories, or something quite like them, will eventually permit a full understanding of all of the fundamental interactions seen in nature, except perhaps gravity.

Such an understanding is still a long way off and it will come, if at all, only when our facility in using and thinking within the field theoretic formalism has advanced considerably beyond the present level. (I am thinking, for example, of the problem of quark confinement—plainly a non-perturbative effect which will need considerable technical progress to elucidate.) It is therefore a pleasure to welcome an excellent book by Charles Nash which develops some of the fundamentals of field theory with enough detail to teach the serious student how to undertake his or her own calculations. Most of the book is devoted to the  $\lambda\phi^4$  theory and quantum electrodynamics (QED), rather

than the non-abelian gauge theories (Weinberg-Salam and QCD) which launched the revolution. This is all to the good, as it permits the assimilation of unfamiliar techniques without the encumbrances of the new theories.

The first *sine qua non* of the new order is functional differentiation and integration, which is illustrated by the usual Gaussian integral. (Is this the *only* functional integral anyone has done?) Nash takes us gently through this, and even consoles us should we feel "a little perplexed or confused". "This is quite normal". Functionals on a Grassmann algebra are also considered. Next there is an extensive coverage of dimensional regularisation, a covariant and gauge invariant method for handling the infinite quantities which inevitably arise when evaluating radiative corrections in these theories. This is used to verify the Ward Identity and to compute the renormalisation constants in QED. The final chapter touches on the asymptotically free gauge theories, the Callan-Symanzik equation, and so on. However, the coverage is less full than that of the earlier material, and there is no treatment of spontaneous symmetry breaking in non-abelian theories. If these attractive theories do withstand further rigorous experimental tests, it is to be hoped that the author will be moved to write a companion volume dealing with these topics in the same detail. In the meantime the present book will certainly provide a lastingly useful and relatively painless introduction into a highly technical field.

D. Bailin

D. Bailin is Lecturer in Theoretical Physics at the University of Sussex, Brighton, UK.

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April 1979 456 pages  
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## Amino acid analysis

*Amino Acid Determination: Methods and Techniques.* Second edition. Edited by S. Blackburn. Pp. 367. (Marcell Dekker: New York and Basel, 1978.) Swiss Fr. 82.

THIS will be a most useful book which should find its way on to the shelves of every amino acid analyst. Old hands will find new and valuable information, and beginners will find a thorough introduction to the field. Perhaps its best features are the extensive and thorough bibliography which accompanies each chapter and the author index, covering over 1,000 authors. Nevertheless, and despite its subtitle, this is *not* a laboratory manual in the sense of providing recipes and precise details of procedural matters.

There are 13 chapters of which the editor has written a short introduction and recent history (chapter 1), an equally short tailpiece (chapter 13) and three other chapters. Of the remaining eight chapters, five are by George W. Robinson and the others by experts in their particular areas.

Chapter 2 (by Blackburn) describes the preparation and hydrolysis of samples before analysis and is a useful survey of the methods and pitfalls in this necessary preliminary. There follow four chapters (3–6) by Robinson on the standard ion-exchange methods used in modern automatic analysis, dealing with the theory, development, and practical aspects such as resins, buffers and detection systems. Chapter 7, entitled "Automatic Analysers", is the least satisfactory in the book. Admittedly, information on makes of instruments and their capabilities and defects is necessarily ephemeral material but it is nevertheless very valuable information for anyone undertaking the purchase of a new analyser. This chapter could therefore have said much more about the strengths and weaknesses of the different instruments and their makers. For example, although the editor mentions the Rank-Hilger Chromospek in chapter 1 as possessing some "novel features", neither the instrument nor its features are mentioned in chapter 7. In fact, novel though they may be, there are serious disadvantages that could well have been discussed and compared with the pros

and cons of other makers' instruments.

Blackburn's chapter 8 on "Gas Chromatography" will be found useful by GC buffs no doubt, but for a method which, in the words of the Editor in chapter 1, "has failed to displace the amino acid analyser" this chapter is extraordinarily long (78 pages compared to a total of only 69 in chapters 3–7 on the analyser). This may be necessary to do the method justice, as it does have specialist uses; but the balance seems wrong.

The use of computers in amino acid analysis is well covered in chapter 9, and chapter 10 is a rag-bag of miscellaneous procedures and applications containing a useful section on dansyl amino acids. An unexpected blessing, at least for those with medical interests, is chapter 11 on "Physiological Fluids". This is a real mine of densely packed information. Finally, chapter 12 is a useful account of the relatively new techniques using pyridoxal derivatives of amino acids.

W. Ferdinand

W. Ferdinand is Senior Lecturer in Biochemistry at the University of Sheffield, UK.

## Viking missions

*To the Red Planet.* By Eric Burgess. (Columbia University Press: New York, 1978.) \$24.95.

ALTHOUGH all the plates and other illustrations in this book are in black and white, this is more than compensated for by the actual choice of graphic material and the informed, balanced and stimulating text. The opening chapters give a lucid account of the historical background and current state of knowledge regarding the planet Mars, the biophysical and biochemical bases of life as we know it, and how these topics relate to the objectives of the Viking missions.

Passages devoted to spacecraft instrumentation and hardware, which appear mainly in chapter 4, are treated in the contexts of the scientific rationale and mission planning. The emphasis here, as throughout the book, is sufficiently human in orientation that one does not lose sight of the involvement, the thoughts and reactions, of the project scientists; and the author pays due regard to maintaining the requisite attention to scientific and technical detail.

Burgess arranges his facts well and combines them with lively narrative and wit, not born of offhandedness nor

any trace of cynicism, but of the humour flexed by an author who really is in control of his material and who obviously feels a pressing need to communicate this to a wide spectrum of readers.

Each point of discussion is given a fresh paragraph, assisting the overall clarity, but a few sub-headings would not have gone amiss, particularly where the Viking lander experiments are described in chapter 7 and where conclusions are drawn in the final chapter. But this is not a serious detraction. Possibly through modesty the author may not have regarded his work as a book of reference. In view of the selection of topics and pictures discussed, and the high standard of his own exposition, it certainly could be regarded as such.

Burgess has undertaken a difficult task and performed it well; readers of the book will undoubtedly be rewarded. If they happen to be members of the public at large or specialists in planetary science, it will go a long way towards broadening their appreciation of the considerable amount of interdisciplinary thought, effort and coordination which has contributed to the success of the Mars Viking missions.

D. J. Telfer

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## Ageing of higher organisms

*Principles of Mammalian Ageing.* Second edition. By R. R. Kohn. Pp. 240. (Prentice-Hall: Englewood Cliffs, New Jersey, and Hemel Hempstead, UK, 1978.) \$19; £13.85.

GERONTOLOGY was born in optimism. It threatens to founder in verbiage. Papers and books are now appearing in quantity, while declining in quality, drowning the few really important studies in this field. Robert Kohn's book was there at the beginning of the boom, and it stands aside from the information explosion. Despite certain problems, this new edition restores to us a compact, well-written and refreshingly intelligent introductory text on the ageing of higher organisms.

The book begins by exploring the progressive crystallisation and toughening of inorganic materials as an analogy to illustrate ageing. It is an analogy which some may find weak and misleading, for ageing is manifest by increasing disorder rather than order. Nevertheless it serves the author's purpose, for he moves on to an excellent summary of the ageing phenomena in the extracellular materials of the body. The following sections deal with ageing within cells, briefly covering the evidence for age-related changes in the dynamic renewal of cellular constituents and for long-term biochemical deterioration. This is followed by a carefully ordered chapter on ageing of cell populations, which classifies cells according to their degree of continuing cell division within tissues. The rest of the book is concerned with the ageing of animals and here the author makes a good job out of the onerous task of summarising a highly complex and contradictory field.

The problem with the book is the author's penchant for selecting two or three seemingly contradictory papers as representative of major fields of ageing research, which then allows him to conclude that this work is inconclusive. For example, there is excellent evidence that dividing cell populations such as mammary gland cells, antibody-producing cells or haematopoietic cells show some age changes during the lifespan of the individual and will eventually cease division, although they are capable of outliving the organism from which they are derived. The author fails to mention significant work in this field and uses sparse studies to support a 'no-evidence' conclusion. He does the same with the exciting new field of the analysis of errors in macromolecules of senescent cells, with work on histones, on the question of protein

turnover, repair capacity, DNA synthesis and the genetics of ageing as a whole. A more penetrating analysis would have shown him that work which at first reading seems contradictory is in fact complementary, and would have enabled him to pick out the key papers in each field. This would have allowed him to draw constructive conclusions in each area, thus enriching the book.

The author's unwillingness to make conclusions derives in part from a certain failure to properly grasp molecular, genetic and evolutionary concepts as a whole. He remakes the classic *faux pas* that ageing exists because "old, non-reproductive animals compete with younger animals for food", which is the same as saying that ageing exists because of ageing. Besides this, scientists usually have some inner vision which guides their work, especially in such a complex and

exploratory field as ageing research. To some, ageing is a process of headlong over-differentiation. To others it is an ineluctable entropic deterioration of biological information. Krohn seems to see ageing as a kind of crystallisation or fossilisation of biological materials. Each man to his own. But it is important to be able to understand other researchers' ways of looking at the problem.

Yet it is easy to criticise a scientific text on the basis of selectivity. These criticisms do not alter the fact that the book holds together very well. Now that the number of papers on ageing is somewhere at the five figure level, a coherent and thoughtful introductory text such as this is no mean feat.

**Stephen J. Fulder**

*Stephen J. Fulder is about to take up a research post at the Brookdale Institute of Gerontology, Jerusalem, Israel.*

## Transfer RNAs

*Transfer RNA.* Edited by S. Altman. Pp. 356. (MIT Press: Cambridge, Massachusetts, and London, 1979.) \$30; £21.

THE concept of tRNAs as the essential links between nucleic acid and protein, has for over twenty years been a central theme of molecular biology in general and studies on protein biosynthesis in particular. It is now some thirteen years since the 'state of the art' was comprehensively reviewed in a Cold Spring Harbor Symposium volume. Subsequent years have witnessed a veritable explosion in the literature, not only in classical areas of tRNA research but also in new ones such as analysis of tRNA genes, tRNAs as primers of reverse transcriptase activity, and determination of tRNA tertiary structure.

It is thus more than timely for an overview of all these aspects of tRNA function. This book succeeds admirably in this task, by providing a number of reviews, all by leading authorities in the field. The introduction (by Zachau) serves to establish the subject in a historical perspective, as well as reminding us of the many questions still left unanswered. The impact of our knowledge of the tertiary structure of just one tRNA is well illustrated throughout the book, and not only by the chapter on its crystal structure and immediate implications (by Kim). In particular, Clark's chapter collating features of primary, secondary and tertiary structure provides a fascinating account of the generalisations and clues to function at the molecular level provided by the yeast phenylalanine tRNA crystal

structure. The essay by Crothers and Cole on conformational changes of tRNA in solution serves to provide a physically based viewpoint complementary to descriptions of structure in the crystalline state.

The contribution of Iglio and Cramer on tRNA synthetases and their substrate interactions, elegantly describes the current status in this area, to which Cramer's group have themselves made many significant contributions. The beginnings of understanding at the molecular level are also discussed by Pongs in a chapter largely concerned with ribosomal and mRNA recognition in relation to protein synthesis. Both these chapters clearly indicate the important directions to pursue in the future, difficult though they will be.

Other sections in this book are more concerned with biological topics. Thus, Altman himself reviews the area of tRNA biosynthesis, and with Körner and Feinstein he discusses tRNA-mediated suppression. Many other roles of tRNA are reviewed by LaRossa and Söll, Nishimura, in a chapter on modified nucleosides and isoaccepting tRNAs, suggests that many of these regulatory roles involve modified residues, in as yet unrevealed ways.

Throughout this book the sense of excitement still pervading many areas of transfer RNA study, come across very clearly. It can thus be recommended as a worthwhile and stimulating read for graduate and even advanced undergraduate students in molecular biology as well as for those workers more actively involved in the subject.

**Stephen Neidle**

*Stephen Neidle works in the Department of Biophysics, King's College, University of London, UK.*



# announcements

## Meetings

27 May–1 June, **14th World Gas Conference of the International Gas Union**, Toronto (F. H. Ellins, Canadian Gas Association, 55 Scarsdale Road, Don Mills, Ontario M3B 2R3).

29 May–1 June, **Radioimmunoassay**, Brescia (Centro Congressi del Garda, Villa Alba, Gardone Riviera (Brescia) Italy).

8–10 June, **Multidisciplinary Aspects of Brain Tumour Therapy**, Brescia (Centro Congressi del Garda, Villa Alba, Gardone Riviera (Brescia) Italy).

12 June, **Weed Workshop '79**, Oxford (ARC Weed Research Organisation, Begbroke Hill, Yarnton, Oxford, UK).  
13–15 June, **Ultrasoft X-ray Microscopy**, New York (Conference Director, The New York Academy of Sciences, 2 East 63rd St, New York, New York 10021).

18–20 June, **Anion Transport in Epithelia, Isolated Cells and Cell Membranes**, New York (Conference Director, The New York Academy of Sciences, 2 East 63rd St, New York, New York 10021).

10–15 June, **Specialised Membrane Functions**, Rome (Prof. L. Masotti, Istituto di Chimica Biologica, Ospedale Maggiore, Via Cramsci 14, Parma, Italy).

18–22 June, **20th Advanced Course of Clinical Oncology**, Milan (Lucia Manfredi, Ufficio Attività Didattiche, Istituto Nazionale Tumori, Via G. Venezian, 1 20133 Milan, Italy).

18–21 June, **Metabolic Effects of Alcohol**, Milan (Nutrition Foundation of Italy, Via S. Pietro all'Orto 17 20121 Milan, Italy).

2–6 July, **Automatic Control in Space Symposium**, London (The Institute of Measurement and Control, 20 Peel Street, London W8, UK).

17–24 July, **2nd International Congress of Systematic and Evolutionary Biology**, Vancouver (University of British Columbia, Vancouver, Canada).

24–26 July, **6th International Symposium of Synthesis in Organic Chemistry**, Cambridge (Dr John Gibson, The Chemical Society, Burlington House, London W1, UK).

25–27 July, **Food Control in Action Symposium**, Guildford (Dr D. A. Rosie, Department of Food Science and Applied Biology, Polytechnic of the South Bank, Borough Road, London SE1, UK).

20–25 August, **3rd International Con-**

**ference on Surface and Colloid Science**, Stockholm (The Swedish Institute for Surface Chemistry, c/o Stockholm Convention Bureau, Strandvagen 7 c, S-114 56 Stockholm, Sweden).

2–6 September, **7th International Meeting of the International Society for Neurochemistry**, Jerusalem (Prof. S. Gatt, Department of Biochemistry, Hadassah Medical School, Hebrew University, P.O. Box 1172, Jerusalem, Israel).

2–7 September, **Regulation and Function of Neural Peptides**, Brescia (Marco Trabucchi, M.D., Ente Universitario Lombardia Orientale, Istituto di Farmacologia e Terapia, Via Valsabbina, 19, 25100-Brescia, Italy).

2–7 September, **10th International Hot Atom Chemistry Symposium**, Loughborough (Dr D. J. Malcome-Lawes, Chemistry Department, Loughborough Institute of Technology, Loughborough, Leicestershire, UK).

3–6 and 6–7 September, **Conference on Bio-medical Applications of Lasers and Lasers in Photomedicine and Photobiology**, Florence (Prof. R. Pratesi, Laboratorio di Elettronica Quantistica, Via Panciatichi 56/30, Firenze, Italy).

3–6 September, **EMAG '79**, Brighton (Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

3–7 September, **Mass Spectrometry**, Swansea (Mrs S. Leclercq, The Chemical Society, Burlington House, Piccadilly, London W1, UK).

4–7 September, **Viral Enteritis in Humans and Animals**, Yvelines (Dr R. Scherrer, Station de Recherches de Virologie et d'Immunologie, 78850 Thiverval-Grignon, France).

9–14 September, **7th International Meeting of the International Society for Oncodevelopmental Biology and Medicine**, Guildford (A. M. Neville, Ludwig Institute for Cancer Research, The Haddow Laboratories, Sutton, Surrey, UK).

10–12 September, **4th Annual Symposium of the Uranium Institute**, London (Ms J. Murray, Uranium Institute, New Zealand House, Haymarket, London SW1, UK).

10–12 September, **Biennial Polymer Physics Conference**, Weybridge (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

10–20 September, **International Symposium on Rift Zones of the Earth**, Jerusalem (Dr Zvi Garfunkel, Depart-

ment of Geology, Hebrew University of Jerusalem, Jerusalem, Israel).

10–13 September, **12th Annual Quantum Chemistry Conference**, Oxford (Dr P. J. Grout, Theoretical Chemistry Department, 1 South Parks Road, Oxford).

10–15 September, **International Anaesthesia Postgraduate Course**, Vienna (Vienna Medical Academy, Alser Strabe 4, A-1090 Vienna, Austria).

10–13 September, **Cellular Responses to Mutagens and Carcinogens**, Brighton (Prof. R. J. Cole, School of Biological Sciences, University of Sussex, Brighton, UK).

10–14 September, **EUCHEM Conference on Organic Free Radicals**, Cirencester (Dr J. F. Gibson, The Chemical Society, Burlington House, London W1, UK).

10–14 September, **Management Studies for R & D Chemists 1979**, Slough (Mrs S. Leclercq, The Chemical Society, Burlington House, London W1, UK).

10–14 September, **Inorganic Biochemistry**, Oxford (Mrs S. Leclercq, The Chemical Society, Burlington House, Piccadilly, London W1, UK).

16–21 September, **30th Annual Session American Association for Laboratory Animal Science**, Atlanta (Mr J. J. Garvey, AALAS, 2317 W. Jefferson Street, Suite 208, Joliet, Illinois 60435).

16–26 September, **Advances in Radiation Protection and Dosimetry and Medicine**, Sicily (Prof. V. Perez-Mendez, Building 50, Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720).  
17–21 September, **Advances in Crop Production and Crop Protection**, Reading (Dr J. M. Thresh, Association of Applied Biologists, East Malling Research Station, Maidstone, Kent, UK).

18–20 September, **2nd World Filtration Conference**, London (Dr Albert Rushton, University of Manchester Institute of Science and Technology, Manchester, UK).

19–21 September, **4th National Quantum Electronics Conference**, Edinburgh (Dr B. S. Wherrett, Physics Department, Heriot-Watt University, Edinburgh, UK).

1–4 October, **B Cell Differentiation and Idiotypes**, Paris (Inserm Conferences, 101 rue de Tolbiac, 75645 Paris Cedex 13, France).

1–5 October, **15th Congress of the International Union of Game Biologists**, Dublin (Fergus O'Gorman, 44 Northumberland Road, Dublin 4, Ireland).

20–26 September, **World Book Fair on Telecommunications and Electronics**, Geneva (Mr A. G. El-Zanati, 18 quai Ernest-Ansermet, CH-1211 Geneve 4, Switzerland).

23–26 September, **International Symposium on Inborn Errors of Metabolism in Humans**, Interlaken (Mrs Sonja Wyss, Medizinisch-chemisches Institut, Universität Bern, Buhlstrasse 28, CH-3000 Bern 9, Switzerland).

23–27 September, **Gene Transfer and Functional Analysis in Somatic Cells**, Paris (Inserm Conferences, 101 rue de Tolbiac, 75645 Paris Cedex 13, France).

24–28 September, **Growth and Properties of Metal Clusters: Applications to Catalysis and the Photographic Process**, Villeurbanne-Lyon (Société de Chimie Physique, 10 Rue Vaquelin, Paris 5).

24–28 September, **3rd International Reunion for the History of Nautical Science and Hydrography**, London (The Conference Officer, National Maritime Museum, Greenwich, London, UK).

25 September, **Materials Selection for Wear Resistance**, Uxbridge (The Meetings Secretary, The Institution of Metallurgists, Northway House, Whetstone, London N20, UK).

25–27 September, **Organization of Macromolecules in the Condensed Phase**, Cambridge (Mrs Y. A. Fish, Faraday Division, The Chemical

Society, Burlington House, Piccadilly, London W1, UK).

25–26 September, **Hydrocarbons in Biotechnology**, Canterbury (Ms I. McCann, Conference Department, Institute of Petroleum, 61 New Cavendish Street, London W1, UK).

26 September, **Computer Modelling in Ultrasonics**, London (Dr L. J. Bond, Physics Department, City University, Northampton Square, London EC1, UK).

27–28 September, **Workshop on The Uses of Oestrogens as Carriers of Cytotoxic Agents in Hormone Receptive Tumours**, Diepenbeek (Dr J. Raus, Dr L. Willems-Institut, Universtaie Campus, B-3610 Diepenbeek, Belgium).

28–29 September, **Steroid Induced Uterine Proteins**, Marburg (Dr M. Beato, Inst. Physiol. Chemie, 3550 Marburg, Germany).

7–11 October, **Genetic Control and Diseases**, Paris (Inserm Conferences, 101 rue de Tolbiac, 75645 Paris Cedex 13, France).

22–24 October, **19th Annual Hanford Life Sciences Symposium**, Richland (Mrs J. A. Rising, Biology Department, 331 Building, Batelle, Pacific Northwest Laboratories, Richland, WA 99352).

30 October–2 November, **Separation Science and Technology for Energy Applications**, Gatlinburg (Mr A. P.

Malinauskas, Oak Ridge National Laboratory, PO Box X, Oak Ridge, Tennessee 37830).

8–10 November, **International Symposium on Trisomy 21**, Rapallo (Prof. M. Fraccaro, C.P. 217, I-27100 Pavia).

8–10 November, **12th Danube Symposium on Neurological Sciences**, Innsbruck (Vienna Medical Academy, Alser Strabe 4, A-1090 Vienna, Austria).

8–10 November, **Albert Einstein as an Intercultural and Interdisciplinary Phenomenon: His Influence in all Fields of Thought**, New York (University Centre for Cultural and Intercultural Studies, Hofstra University, Hempstead, New York 11550).

12–16 November, **World Conference 3 on Breeding Endangered Species in Captivity**, San Diego (Ms J. Hammer-shoy, Conference Coordinator, San Diego Zoo, PO Box 551, San Diego, California 92112).

23–24 November, **Latest Therapy of Urethral Stricture in Male; Indication, Technique, Postoperative Care and Late Results with Staghorn Calculi**, Vienna (Vienna Medical Academy, Alser Strabe 4, A-1090 Vienna, Austria).

27–30 November, **On Protection of Workers against Noise**, Dresden (Organisationsburo, Postchließfach 105, DDR-8020, Dresden).

## Reports & publications

### Other countries—March

United States Department of the Interior: Geological Survey. Professional Paper 907-E: The Potential for Porphyry Copper-Molybdenum Deposits in the Eastern United States. By Robert Gordon Schmidt. Pp. v+31. Professional Paper 1060: Deformation of the Roberts Mountains Allochthon in North-Central Nevada. By James G. Evans and Ted G. Theodore. Pp. iii+18+2 plates. (Washington, DC: US Government Printing Office, 1979.) [153]

Economic and Social Commission for Asia and the Pacific. Committee for Co-ordination of Joint Prospecting for Mineral Resources in Asian Offshore Areas (CCOP). Technical Bulletin Vol. 12: Contribution to Knowledge of Tectonics and Mineral Resources in East Asia. Pp. v+108. (Tokyo: Overseas Geology Office, Geological Survey of Japan, 1978.) [163]

Clinton Laboratories, Santa Monica, CA. Technical Papers. No. 1: Interconversion of Enzyme Units. By Sylvan M. Sax. Pp. 24. No. 2: Use of Indicator Enzymes for Measurement of Endogenous or Enzyme-Liberated Ammonia. By Dr. David M. Goldberg. Pp. 32. No. 3: SI Units in the Clinical Laboratory. By Dr. Sylvan M. Sax. Pp. 50. (No. 1 \$4; No. 2 \$5; No. 3 \$6.) (Santa Monica, CA: Clinton Laboratories, 1805 Colorado Avenue, 1972, 1978 and 1979.) [163]

Rand 1977–1978. Pp. viii+136. (Santa Monica, CA: The Rand Corporation, 1700 Main Street, 1939.) [163]

International Atomic Energy Agency, Vienna. Technical Reports Series. No. 192: Environmental Isotopes Data No. 6—World Survey of Isotope Concentration in Preceipitation (1972–1975). (Report from a Network Organised by the International Atomic Energy Agency, in co-operation with the World Meteorological Organization and Co-operating National Laboratories.) Pp. xix+187. (Vienna: International Atomic Energy Agency, 1979.) US\$16 [203]

*Journal of Immunopharmacology*, Vol. 1, No. 1, 1978/1979. Executive Editor: Alan M. Reynard. Pp. 1–125. Subscription rate for Vol. 1, 1978/79 \$40 (prepaid). Special discounted rate for individual subscriptions \$20 per volume. (New York: Marcel Dekker Journals, PO Box 11305, Church Street Station, 1978.) [213]

Scripps Institution of Oceanography, 1978. 75th Anniversary Edition. Pp. 66. (San Diego, CA: Scripps Institution of Oceanography, University of California, 1979.) [213]

Lincoln Laboratory, Massachusetts Institute of Technology. Seismic Discrimination, 30 September 1978. (Semiannual Technical Summary.) Pp. viii+74. (Lexington, Mass.: Lincoln Laboratory, MIT, 1979.) [213]

Ninth Report of the Joint Panel on Oceanographic Tables and Standards, Unesco, Paris, 11–13 September

1978. (Sponsored by Unesco, ICES, SCOR, IAPSO.) Pp. 32. (Paris: Unesco, 1979.) [213]

World Health Organisation. Technical Report Series No. 633: Training and Utilization of Auxiliary Personnel for Rural Health Teams in Developing Countries—Report of a WHO Expert Committee. Pp. 35. (Geneva: WHO; London: HMSO, 1979.) Sw.fr.5. [223]

United States Department of the Interior: Geological Survey. Bulletin 1456: Mercury Deposits in Turkey. By M. Yildiz and Edgar H. Bailey. Pp. v+80. (Washington, D.C.: U.S. Government Printing Office, 1978.) [223]

Smithsonian Contributions to Zoology. No. 245: Gammaridean Amphipoda of Australia, Part III: The Phoxocephalidae. By J. Laurens Barnard and Margaret M. Drummond. Pp. viii+551. (Washington, DC: Smithsonian Institution Press, 1978. For sale by US Government Printing Office.) [233]

Eastman Kodak Company, 1978 Annual Report. Pp. 40. (Rochester, New York: Eastman Kodak Company, 1979.) [233]

Report of the Finnish Geodetic Institute. 78:8: Ranging Precision of the Finnish Satellite Laser Range Finder. By Juhani Kakkuri, Ossi Ojanen and Matti Paunonen. Pp. 11. 79:1: On the Analysis of the Return Pulse of the Satellite Laser. By Ossi Ojanen. Pp. 33. (Helsinki: Geodettiin Laitos, Pasilankatu 43 A, 1978 and 1979.) [233]

Why be Quantitative About Radiation Risk Estimates? By Sir Edward E. Pochin. (The Lauriston S. Taylor Lecture Series in Radiation Protection and Measurements, No. 2.) Pp. 36. (Washington, DC: National Council on Radiation Protection and Measurements, 7910 Woodmont Avenue, 1978.) \$6. [263]

International Agency for Research on Cancer. Annual Report, 1978. Pp. 185. (Lyon, France: International Agency for Research on Cancer, 1978.) Sw.fr.12. [263]

Bulletin of the American Museum of Natural History. Vol. 162, Article 3: The Jurassic Turtles of North America. By Eugene S. Gaffney. Pp. 91–136. (New York: American Museum of Natural History, 1979.) \$3. [263]

Zenith Radio Corporation. Annual Report, 1978. Pp. 20. (Glenview, Illinois: Zenith Radio Corporation, 1979.) [263]

Smithsonian Contributions to Zoology. No. 279: Bredin-Archbold-Smithsonian Biological Survey of Dominica. The Superfamily Scarabaeoidea (Coleoptera). By Oscar L. Cartwright and Fortuné E. Chalumeau. Pp. iv+32. No. 286: Fishes of the Genus *Eviota* of the Red Sea with Descriptions of Three New Species (Teleostei: Gobiidae). By Ernest A. Lachner and Susan J. Karnella. Pp. iii+23. (Washington, DC: Smithsonian Institution Press, 1978. For sale by US Government Printing Office.) [263]

Annals of the Transvaal Museum. Vol. 31, No. 9: Neptulidae of Southern Africa—a Taxonomic

Revision of the Genus *Stigmella* Schrank (Lepidoptera: Monotrypa). By M. J. Scoble. Pp. 88–130+1 plate. Vol. 31, No. 10: Ecological Distribution of the Mammals of the Transvaal Vertebrata (Mammalia). By I. L. Rautenbach. Pp. 131–156. Vol. 31 No. 11: Three Species of Microchiropteran Bats Recorded for the First Time from the South-West Cape Biotic Zone. By I. L. Rautenbach and J. A. J. Nel. Pp. 157–164. Vol. 31, No. 12: Dental Abnormalities in *Crocodylus mariqueensis* (A. Smith, 1844) (Mammalia: Soricidae). By N. J. Dippenaar. Pp. 165–168. Vol. 31, No. 13: A New *Glossiphonia* Species from South Africa (*Hirudinea*: Glossiphoniidae). By J. H. Oosthuizen. Pp. 169–176. Vol. 31, No. 14: A New Limestone Cave Breccia from Vlakplaats Near Pretoria. By E. S. Vrba and D. C. Panagos. Pp. 177–184. Vol. 31, No. 15: A Fossil Felid Femur Formerly Considered to Be Hominid (Mammalia: Felidae). By Ivan Suzman. Pp. 185–188. Vol. 31, No. 16: Observations of the Natural History of Perringuey's Adder *Bitis peringueyi* (Boulenger) (Reptilia: Viperidae). By Michael D. Robinson and David A. Hughes. Pp. 189–193+3 plates. (Pretoria: Transvaal Museum, 1978.) [273]

*Loess Letter*, No. 1, April 1979. (Informal newsletter of the Western Pacific Working Group of the INQUA Loess Commission.) Pp. 8. (Lower Hutt, New Zealand: Ian Smalley, DSIR, Soil Bureau, 1979.) [273]

Annals of the South African Museum. Vol. 77, Part 6: Cretaceous Faunas from Zululand and Natal, South Africa. The Ammonite Superfamily Haplocerataceae Zittel, 1884. By William James Kennedy and Herbert Christian Klingner. Pp. 85–121. Vol. 77, Part 7: A New Genus and Species of Solenoceridae (Crustacea, Decapoda) from South-East African Waters. By Antonio J. de Freitas. Pp. 123–131. Vol. 77, Part 8: Type Specimens of Hydrozoa (Coelenterata) in the South African Museum. By N. A. H. Millard. Pp. 134–150. (Cape Town: South African Museum, 1979.) [273]

United States. National Academy of Sciences: National Research Council. Radiochemistry of Bismuth. By Kashinath S. Bhatki, and Bhabha Atomic Research Centre, Trombay, Bombay, India. Pp. vi+142. (Nuclear Sciences Series.) (Springfield, Virginia: National Technical Information Service, US Department of Commerce, 1977.) \$4.75. [273]

Almanac for Computers for the year 1979. (Washington, DC: Nautical Almanac Office, United States Naval Observatory, 1979.) [273]

Smithsonian Contributions to Paleobiology. No. 38: Tertiary and Quaternary Brachiopods from the Southwest Pacific. By G. Arthur Cooper. Pp. iv+23. (Washington, DC: Smithsonian Institution Press, 1978. For sale by US Government Printing Office.) [273]

South African Institute for Medical Research. Annual Report, 1977. Pp. 115. (Johannesburg: South African Institute for Medical Research, 1979.) [273]

Swedish Institute. Current Sweden, No. 212: Swedish Data Policy. By Jan Freese. Pp. 11. (Stockholm: Swedish Institute, Box 7434, 1979.) [293]

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## APPOINTMENTS VACANT

### STRUCTURAL MOLECULAR BIOLOGIST

Position now available in the Biology Department of Brookhaven National Laboratory for a structural molecular biologist with considerable experience in instrumentation in the field of small angle scattering. The appointment is to the Scientific Staff. The scientist will be involved in the development of the Brookhaven National Laboratory Synchrotron facilities for use in small angle scattering experiments.

Applicants should submit a curriculum vitae (including a list of publications) and the names of three references to:

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### THE QUEEN'S UNIVERSITY OF BELFAST RESEARCH ASSISTANT

Marine Biology Station, Portaferry

This project involves a study of the effects of light fluctuations on marine phytoplankton photosynthesis and growth. The post, tenable for three years, will be financed by the N.E.R.C. Commencing salary £3,384 on a scale (under review) rising to £3,885 per annum plus U.S.S.

Further particulars may be obtained from Dr G. Savidge, Queen's University of Belfast, Marine Biology Station, Portaferry, Co Down BT22 1PF, Northern Ireland.

Applications, giving the names and addresses of two referees, should be sent to the Personnel Officer, The Queen's University of Belfast, BT7 1NN, Northern Ireland. Closing date: May 31, 1979.

688(A)

### UNIVERSITY COLLEGE DUBLIN DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a post as RESEARCH TECHNICIAN in the above Department. The successful applicant will assist in a special project sponsored by Nordic Cold Storage Ltd., should hold N.C.E.A. or I.M.L.S. qualifications and preferably with subsequent laboratory experience.

Appointment will be on an annual basis for up to three years on a scale:

Technician £71.67 by four increments—£82.35 per week

Senior Technician £85.31 by four increments—£96.78 per week at a level commensurate with qualifications and experience.

Applications giving details of qualifications and experience and the names of two referees should be forwarded before May 31, 1979 to one of the following addresses:

The Secretary, Department of Biochemistry, University College, Belfield, Dublin 4 or Mr W. J. A. Fearn, 1, The Sanctuary, Westminster SW1P 3JT, London, England.

713(A)

### ATMOSPHERIC SCIENTIST

to work in well-equipped laboratory, with a team on the atmospheric planetary boundary layer. The team is involved at a fundamental level in all aspects of the lower troposphere, and work in progress includes airborne measurement studies and theoretical modelling of the surface layer, clouds and precipitation. Requires Ph.D., or equivalent, in related area. Must be skilled with computer programming, numerical methods and mathematical analysis. A good background in physical understanding is important and ability to participate in all aspects of the programme is desirable. The level of appointment is dependent upon ability and qualifications, and opportunities exist for contributing to training graduate students and lecturing. Minimum starting salary \$18,000 plus liberal benefits. Send resume postmarked by June 30, 1979 to Personnel Department, Desert Research Institute, University of Nevada System, P.O. Box 60220, Reno, Nevada 89506. Affirmative Action/Equal Opportunity Employer.

W102(A)

### UNIVERSITY OF CAMBRIDGE DEPARTMENT OF GENETICS RESEARCH ASSISTANT

A position is available, from October 1, 1979, for 3 years, on a research project funded by the Science Research Council, for a research assistant; experience in Drosophila genetics would be an advantage but not essential. Salary at age 24, £3,883, under review.

Further details may be obtained from Dr M. Ashburner to whom applications, with the names of two referees, should be sent to reach him by June 30, 1979, Department of Genetics, Downing Street, Cambridge CB2 3EH.

708(A)

### UNIVERSITY COLLEGE CARDIFF

Applications are invited for the post of

### RESEARCH ASSISTANT

in the Department of PHYSIOLOGY to work on the Physiology of the Nasal Mucosa of the Domestic Pig. The research is supported by an A.R.C. grant for a period of three years, and will involve experiments to study the roles of the autonomic nervous system and endocrine system in the regulation of the nasal mucosa. More information about the project will be provided by Dr R. Eccles, Department of Physiology, University College, P.O. Box 78, Cardiff CF1 1XL. Salary range: Within R. & A. 1A £3,883 to £4,882 per annum. Duties to commence September 1979.

Applications (two copies), together with the names and addresses of two referees, should be forwarded to the Vice-Principal (Administration) and Registrar, University College, P.O. Box 78, Cardiff CF1 1XL. Closing date June 7, 1979. Reference 1979.

753(A)

### THE BRITISH COUNCIL

invites applications for the following posts:

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2. Lecturer in Rural Sociology (Brazil)

Federal University of Paraíba, Joao Pessoa (required for August 1979)

To lecture to undergraduate and postgraduate students and for post (2) to develop research activities.

Qualifications: PhD with at least 2 years' university teaching experience or MSc. with 5 years' relevant experience (10 years preferred). Age range over 30. A knowledge of or willingness to learn Brazilian Portuguese is necessary. Salary: Cr\$33,000 to Cr\$41,000 per month according to qualifications. (Cr\$49.04 equals £1 at 24/4/79). Benefits: 2-year guaranteed contract.

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698(A)



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This is an opportunity to become involved in a research programme seeking fundamental information on which to base a new generation of packaging films.

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There will be opportunities for publication on some of the theoretical aspects of the work, and for other aspects it is expected the Company will seek patent coverage.

Applicants must be self-motivating and have a first class degree plus relevant research experience in the academic or industrial fields.

An attractive salary is offered and the conditions of service are those normally associated with a progressive company. These include extensive technical library facilities and a contributory staff pension scheme. Assistance with relocation expenses can be given where necessary.



Please apply in writing or by telephone to Mr R. C. Rudd, Group Personnel Department, British Cellophane Ltd., Bath Road, Bridgwater, Somerset TA6 4PA. Tel: 0278 4321 ext. 558.

749(A)



**NATIONAL INSTITUTE FOR RESEARCH IN  
DAIRYING  
(UNIVERSITY OF READING)**

**NUTRITION DEPARTMENT**

A graduate is required to participate in a programme of fundamental research into the physiological role of trace nutrient binders in milk and other foods. The work will be mainly concerned with the isolation, characterisation and comparative biochemistry of binding substances from milks of different mammalian species, in particular from bovine and human milk. The appointed person will work within a small group studying binding substances and will be expected to initiate and execute experimental work with a minimum of supervision.

Qualifications: First or Upper Second class Honours Degree in biochemistry or recently qualified Ph.D. Applicants with experience in protein chemistry and enzymology will be at an advantage.

Appointment will be as Scientific Officer (£2,839 to £4,415) or Higher Scientific Officer (£4,101 to £5,448) according to qualifications and experience. At least two years' relevant postgraduate experience is required for appointment as H.S.O. Non-contributory superannuation scheme.

Application forms are obtainable from the Secretary, NIRD, Shinfield, Reading RG2 9AT. Quote reference 79/15. 595(A)

**Division of Hormones**

**Postdoctoral  
Scientist**

Required to work on a three-year project as part of a team and to collaborate with other groups engaged on studies of the mechanism of action and secretion of hormones; for studies on the mechanism of coupling between hormone receptors and adenylate cyclase systems in cell membranes, using cell fusion techniques.

A knowledge of biochemical endocrinology, cell membrane systems and/or cell fusion processes would be an advantage.

Salary in accordance with age and experience, on university lecturing scales.

Application form and further details from The Personnel Section, NIBSC, Holly Hill, Hampstead, London NW3 6RB. Tel: 01-435 2232, ext. 200. (Please quote reference number HO/277).

**NIBSC**

National Institute for Biological Standards and Control

**IMMUNOPHARMACOLOGIST**

The Research Unit of Organon Laboratories Limited is an integral part of the Scientific Development Group of Organon International, a major international pharmaceutical company based in the Netherlands.

Applications are invited for this major appointment in our Biochemistry R. & D. Department. This department is responsible for long term research aimed at the development of new drugs for treating chronic inflammatory diseases with emphasis on the involvement of the immune response. This is a challenging post working as part of a young and enthusiastic interdisciplinary team within a rapidly developing area of research.

Suitable candidates will already be working on the immunological aspects of chronic inflammation and have a Ph.D degree with several years postdoctoral experience.

The successful candidate will be expected to maintain and develop his expertise and reputation in the field by attendance at scientific meetings and by publication of his research results.

The initial salary will be based on qualifications and experience and will be commensurate with the responsibilities of the position. Conditions of employment are in keeping with good modern practice and assistance with relocation expenses will be available in appropriate cases.

Applications should be sent to the Personnel Manager, Organon Laboratories Limited, Newhouse, Lanarkshire, ML1 5SH. 730(A)



**INSTITUTE OF DENTAL SURGERY  
BRITISH POSTGRADUATE MEDICAL FEDERATION  
UNIVERSITY OF LONDON**

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**HEAD OF DEPARTMENT/SENIOR LECTURER**  
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Previous dental experience is not essential, but applicants should have a postgraduate degree in the medical/dental or biological sciences and research experience. If appropriate, the successful candidate will be given honorary consultant status by the associated Eastman Dental Hospital.

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(Salary awards pending on scales).

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**LIVERPOOL POLYTECHNIC  
Department of Chemistry and Biochemistry  
RESEARCH ASSISTANT  
£2511—£2697**

Applications are invited for an L.E.A. research assistant to work with Dr. D. Billington on biochemical mechanisms involved in mammalian bile formation, with particular reference to hepatic bile salt transport.

Application forms and further particulars may be obtained from The Personnel Office, Richmond House, 1 Rumford Place, Liverpool L3 9RH, or by telephoning Dr. Billington on 051-207 3581 Ext. 32.

Please quote reference L.P.284.

724(A)

**CSIRO****AUSTRALIA**

# Division of Computing Research

Canberra A.C.T.

**General Manager (4 positions)****Section Leader (6 positions)****Research Scientist (several positions)****Experimental Officer (System Specialist) (4 positions)****General**

The Commonwealth Scientific and Industrial Research Organisation (CSIRO) is a statutory authority established in 1949 by act of the Australian Parliament. The functions of CSIRO include carrying out and facilitating the application and utilisation of scientific research to assist Australian industry, furthering the interests of the Australian community, and contributing to the achievement of Australian national objectives.

The Division of Computing Research (DCR) is responsible for the research, development, maintenance and operation of systems and equipment used by the national computing facility, named CSIRONET. The facility serves CSIRO, Government Departments and Instrumentalities, and other approved users. CSIRO, through DCR, is also called on to advise Government and others on computing policy and requirements. It has an annual revenue in excess of \$6 million, and employs about 140 staff. The functions of DCR have recently been reviewed, leading to the restructuring of the Division into four Branches to meet the computing needs of its users as these requirements increase in quantity and complexity and change in direction. Each Branch comprises a number of research and development Sections and/or support Sections.

Each research and development Section comprises a research leader, at least one other research scientist, together with an appropriate support staff. The Sections solely engaged in support or co-ordination are not led by research staff. With few exceptions it is expected that the members of a Section will be located together. Each present or proposed Section is placed in a capital or large provincial city which includes at least one university and other tertiary educational or scientific institution.

Access to the CSIRONET host computers is provided by about 650 interactive and 250 batch devices connected to a packet switching network involving 80 minicomputer nodes. Internode lines operate at speeds up to 48 kilobits per second. During prime shift about 40 megabytes per hour are transmitted through the network. Special purpose nodes are implemented, or planned to provide spool, monitor, sentry, gateway and other distributed functions. A resource sharing network using Network Systems adaptors driving coaxial cables operating at 50 megabits per second is being implemented to serve the host computers. The principal general purpose host at present is a maximum configuration Control Data Cyber 76, supported by 7 GB (gigabytes) of disc storage. A Cyber 17X supported by 2 GB of disc and 32 GB mass storage will be installed for developmental work. A Facom M190 supported by 2.4 GB is used as a research and development vehicle. Special purpose hosts include an Information International COMp80 phototypesetting and (micro) graphics system, a COMTAL/PDP11 image processing system, a VM system controlling a Calcomp automatic tape library, and other development systems.

**Duties:****General Managers**

Under the Chief of the Division, the General Managers are the most senior officers of DCR, and are responsible for policy development, and for the management of the research and development activity of a Branch of the Division.

A General Manager may be required to deputise for the Chief of Division. Frequent interstate travel is necessary.

**The four Branches are:**

1. Computing Systems Branch. Activities include research and development and the maintenance of the Cyber 76 Scope system, the NOS operating system, and VM system, and OSIV F4 system, mass storage systems, and distributed processing systems.

2. Information Systems Branch. Activities include research and development in distributed data base systems, information retrieval, computer security, simulation and system analysis, computational methods, classification methods, program structures and mathematical programming.

3. Peripheral Systems Branch. Activities include research and development of VLSI architecture and other hardware projects, picture processing, graphics, micrographics, the research, development and maintenance of packet switching and resource sharing networks, and of network performance measuring tools.

4. Co-ordination and Support Branch. Responsibilities include the co-ordination of the research, development and system maintenance

activities of the Division. Other activities of this Branch are system documentation, user assistance, applications support, operations, business systems, accounting, administration, site management and the Divisional library.

**Section Leaders**

Under the broad direction of a General Manager, a Section Leader is responsible for the planning and conduct of a particular research and development program, including maintenance of products and systems developed by the Section, and for consultation with users and organization of workshops and seminars.

**Section Leaders are required for:**

1. Information Retrieval (Melbourne)
2. Stochastic Simulation (Sydney)
3. VLSI Architecture (Adelaide)
4. Computational Method (Brisbane)
5. Program Structures (Perth)
6. Mathematical Programming (Hobart)

**Research Scientist**

Is required to initiate and undertake a research and development program in a particular field. Some responsibility for activities of other staff may also be required. Vacancies exist in the above six sections, and also in:

7. Operating Systems (Canberra)
8. Classification Methods (Townsville)
9. Continuous Simulation (Brisbane).

**Experimental Officer (System Specialist)**

Under the broad direction of a General Manager, is the leader of a number of professional and support staff in a Section responsible for the maintenance of a major computing or support system in one of the following fields:

10. Computer security (Canberra)
11. NOS system (Canberra)
12. VM system (Canberra)
13. User services (Canberra)

**Qualifications**

For appointment to positions of General Manager, Section Leader or Research Scientist, a Ph.D. or equivalent qualification in the field of Computing or Information Science is required. Applicants for the positions of General Managers and Section Leaders should have had extensive experience relevant to the position concerned, supported by evidence of management ability at an appropriate level.

For appointment as Experimental Officer, a degree or equivalent qualification in Computing or Information Science is required, together with extensive professional experience relevant to the position concerned.

**Salary**

Successful candidates would be appointed within the following salary ranges:

General Manager: \$A27,790-\$A30,530 p.a. with consideration of appointment at higher level for an outstanding applicant.

Section Leader: \$A23,247-\$A30,530 p.a.

Research Scientist: \$A15,422-\$A22,405 p.a.

Experimental Officer: \$A17,800-\$A21,702 p.a. with consideration of appointment at a higher level for an outstanding applicant with proven management ability of a high order.

**Conditions**

Conditions of service include participation in the Superannuation Scheme, long service and sick leave benefits and four weeks annual leave. Appointee's fares, removal expenses and an allowance until permanently housed may be paid.

**Tenure**

Indefinite or fixed term appointments may be negotiated, both of which carry Superannuation benefits.

**Applications**

Applications in duplicate stating full personal and professional details, and the names of at least two referees, and quoting reference number 900/345 and the position in which interested should reach: The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 9th June 1979.

Applications in USA and Canada should be sent to: The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, N.W., Washington, D.C., 20036, USA.

755(A)



Open to both  
men and women

 **Public Service Commission**  
**Canada** **Fonction publique**  
**Canada**

## MARINE RESOURCES ASSESSMENT SCIENTISTS

Salary: \$16,500 to \$39,517 (depending on  
qualifications)

Fisheries and the Environment  
Fisheries Management  
St. Andrew's, New Brunswick

Two positions are available at the St. Andrew's Biological  
Station, to develop and evaluate resource inventory methods  
for pelagic and groundfish stocks in Eastern Canadian waters  
as part of the stock assessment process.

### SECTION HEAD, RESOURCE INVENTORY (109-025-017)

Ref. No: 79-NCRSO-32-150 (N)

The senior position of the two vacancies is for the leader of the  
Resource Inventory Section which has a staff complement of  
about twenty. In addition to coordinating the development of  
resource inventory programs, the successful candidate will  
manage the fish aging service of the division; will coordinate  
the fish sampling activities and computer services of the  
section; and, will collect data on oceanographic parameters.

### RESEARCH SCIENTIST, FISH SURVEYS (109-037-023)

Ref. No: 79-NCRSO-32-149 (N)

The junior position is for a scientist to conduct research  
primarily on the design and development of pelagic fish  
survey techniques.

### QUALIFICATIONS

Applicants for either of the above positions must possess a  
PhD in quantitative fish biology with specific training in  
statistical methodology. A lesser degree coupled with  
evidence of research experience and productivity which is  
equivalent to a doctorate is also acceptable.

For the junior position, experience in applied fisheries  
research and evidence of ability in program management  
are desirable.

For the senior position, applicants must have published a  
substantial number of scientific papers and be recognized as  
experts in this field at the national level. Experience in program  
management is essential for this position.

Knowledge of English is essential.

### How to Apply

Send your application form and/or résumé to:

D. E. Fraser, Staffing Officer  
National Capital Region Staffing Office  
Public Service Commission of Canada  
Ottawa, Ontario K1A 0M7  
Closing Date: May 31, 1979

Please quote the applicable reference number at all times. 746(A)

## SENIOR BIOLOGIST £5,682—£6,750 p.a.

male or female, with good honours degree in biology, to  
undertake routine macro- and micro-biological exami-  
nations, including appraisals of algal status of raw water  
reservoirs, macro-biological evaluations of reservoir  
marginal areas, of mains infestations and consumer com-  
plaint samples. In addition research and development  
projects are actively encouraged.

## ASSISTANT BIOLOGIST £4,179—£4,476 p.a.

recently qualified graduate, male or female, to assist the  
Senior Biologist. An excellent opportunity for acquiring  
wide-ranging experience within the water supply industry.

Both posts would initially be based at Lichfield, Staffs,  
but will be moving to new laboratories presently being  
constructed at Walsall, West Midlands. Reasonable re-  
location expenses payable.

Please write by May 24, to

The Chief Engineer,  
The South Staffordshire Waterworks Co.,  
50 Shepcote Street, Birmingham B16 8AR. 705(A)



**PLYMOUTH  
POLYTECHNIC**

### RESEARCH ASSISTANTS required for the following areas of study: School of Maritime Studies ASTRONOMY

To analyse data connected with the  
magnetic field of the Milky Way.  
Applicants should have a good honours  
degree in Physics, Mathematics or  
Astronomy. An interest in computing  
and astronomical data analysis is  
essential.

School of  
Environmental Sciences  
LITHOSTRATIGRAPHY,  
BIOSTRATIGRAPHY,  
SEDIMENTOLOGY AND  
PALAEOLOGY OF THE  
UPPER GREENSLAND.  
THE UPTAKE OF POLY-  
NUCLEAR AROMATIC  
HYDROCARBONS IN  
ESTUARINE FOOD WEBS.  
DISPERSAL AND HOST-  
PLANT FINDINGS IN DELTA  
BRASSICAE IN RELATION  
TO AGRICULTURAL  
PRACTICE.  
THE MIGRANT POPULATION  
OF PLYMOUTH IN THE  
19TH CENTURY.  
CARBON AND COKE  
REACTIVITY IN ZINC-LEAD  
BLAST FURNACE PRACTICE.  
STRUCTURAL AND  
GEOTECHNICAL STUDIES  
OF PLYMOUTH LIMESTONE.

Candidates for predoctoral assistant-  
ships should have a good first degree  
in a relevant discipline.

Research Assistants are normally  
required to register for a higher  
degree, although postdoctoral appli-  
cants will be considered. Appointments  
are for a period of two years,  
normally commencing September 1,  
with a possibility of a third year  
(Fixed term contracts). Salary will be  
£3,192 for postgraduates or £3,468  
postdoctoral (or equivalent) with  
annual increments of £138.

Application forms to be returned by  
Friday, June 8, 1979 can be obtained  
with further particulars (please state  
which project) from the Personnel  
Officer, Plymouth Polytechnic, Drake  
Circus, Plymouth PL4 8AA. 701(A)

## UNIVERSITY OF CAMBRIDGE

Applications are invited for the  
office of

### UNIVERSITY LECTURER IN THE DEPARTMENT OF PHARMACOLOGY

from those working in any area of  
experimental pharmacology, and  
applicants with experience in mem-  
brane current fluctuation analysis are  
particularly encouraged.

The initial appointment will be for  
three years from October 1, 1979 with  
the possibility of reappointment to  
the retiring age. The pensionable  
stipend for a University Lecturer will  
be on a scale of £5,367 rising by  
twelve annual increments to £8,257 a  
year (under review), with initial  
placing above the minimum where  
appropriate. There is no grade of  
Senior Lecturer. A grant is made  
towards removal expenses.

Candidates should send 12 copies of  
their application together with the  
names of not more than three referees  
to Mr G. R. ANDERSON, General  
Board, Office, The Old Schools,  
Cambridge CB2 1TT, from whom  
further information can be obtained,  
to arrive not later than June 1, 1979.  
751(A)

## ST. GEORGE'S HOSPITAL MEDICAL SCHOOL (University of London)

### LECTURER/SENIOR LECTURER IN PHARMACOLOGY

The appointment is tenable from  
September 1, 1979. The Department  
of Pharmacology has a strong interest  
in the post-synaptic action of neuro-  
transmitters both in the brain and the  
peripheral nervous system using a  
variety of electrophysiological tech-  
niques. Although preference may be  
given to candidates with an interest  
in biochemical aspects of drug receptor  
interactions or transmitter release,  
strong candidates from all disciplines  
will be considered. Salary ranges  
£3,975 to £8,250 (Lecturer) and £7,980  
to £9,885 (Senior Lecturer). (Both  
ranges under review). In addition  
London allowance £502 per annum.

Further particulars available from  
the Establishment Officer, St George's  
Hospital Medical School, Cranmer  
Terrace, London SW17 0RE, to  
whom applications, with the names of  
three referees, must be sent to arrive  
by June 4. 748(A)



# Section Head— Inhalation Toxicity

## ICI Central Toxicology Laboratory

This Laboratory provides advisory and experimental services to ICI Limited, for the control of toxic hazards in manufacturing processes and in other fields of growing importance, such as the development of pesticides and food additives. Emphasis is placed on improving and assessing methods for studying the biological effect of foreign compounds and upon understanding mechanisms of toxicity. The Provision of toxicological information, prior to product clearance and registration in many countries, is a major activity undertaken by the Laboratory on behalf of the ICI group.

We currently have a vacancy for someone to head our Inhalation Toxicity Section. The job holder will be responsible for managing approximately 18 people who are involved in the provision of a facility to assess any potential health risks through inhalation of chemicals and particulates. The job holder is expected to contribute to the overall management of the Laboratory, and to formulate the correct technical policy for the Section whilst ensuring that results produced are reliable.

The successful candidate will have post-doctoral experience of research on the pulmonary system together with proven ability in managing multi-disciplinary project teams. It is unlikely that any candidate below 30 years of age will have accumulated sufficient experience.

The Laboratory is two miles south of the village of Alderley Edge on the main road (A34) between Manchester and the Potteries on a 300 acre site which the Laboratory shares with ICI's Pharmaceuticals Division. Alderley Edge is less than 20 miles from Manchester and in addition to the well-wooded Cheshire countryside which surrounds it, Alderley is within easy reach of the Derbyshire Peak District, the Lake District and the North Wales Coast. Staff working at Alderley live over a wide area but mainly in the Macclesfield and Alderley Edge/Wilmslow area.

In addition to salary ICI also operate a Productivity Bonus and Profit Sharing Scheme.

Anyone interested in applying for this vacancy (either male or female) should telephone or write (quoting reference IT/SH/SCC) to:-



Miss S. C. Carson, Personnel Officer,  
ICI Ltd., Central Toxicology Laboratory,  
Alderley Park, Nr. Macclesfield,  
Cheshire.  
Phone: Alderley Edge 582711,  
extension 144.

Overseas candidates should only apply if they have a planned visit to the UK within the next two months.  
Closing date for applications: 31st May 1979.

719(A)

### UNIVERSITY OF KHARTOUM—SUDAN

Applications are invited for the following posts in the FACULTY OF EDUCATION:-

#### DEPARTMENT OF

#### CHEMISTRY (Three Posts)

LECTURER in Physical Chemistry  
—Theoretical Chemistry (wave mechanics—theories of bonding—Spectroscopy).

LECTURER in Physical Chemistry  
—Thermodynamics and related fields.

LECTURER in Inorganic Chemistry.

#### DEPARTMENT OF PHYSICS

Four LECTURERS in Classical and Modern Physics.

Salary scale (exclusive of cost of living allowance) £S1,500 to £S3,500 per annum (£S1 equals £1.20 sterling). The British Government may supplement salaries in range £3,894 to £4,542 per annum (sterling) for married appointees (reviewed annually and normally free of tax) and provide children's education allowances and holiday visit passages. Family passages; baggage allowance; superannuation scheme; unfurnished accommodation available; various allowances. Detailed applications (two copies) with curriculum vitae and naming three referees to be sent direct to Acting Personnel Secretary, University of Khartoum, P.O. Box 321, Khartoum, Sudan by June 28, 1979. Applicants resident in the U.K. should also send one copy to: Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 760(A)



**PLYMOUTH  
POLYTECHNIC**

#### SCHOOL OF ENVIRONMENTAL SCIENCES

### LECTURER II ECOLOGY

**SALARY: £4,101 to £6,558**

To organise and take part in undergraduate courses in marine, freshwater and/or terrestrial ecology.

Should be honours graduate in Biology or environmental sciences with a strong ecological background and a Ph.D. or equivalent research experience.

Opportunity to develop special teaching interests in honours degree options and/or M.Sc. in applied Fish Biology. Further research will be encouraged.

Application forms to be returned by **Friday June 8, 1979**, can be obtained with further particulars from the **Personnel Officer, Plymouth Polytechnic, Drake Circus, Plymouth PL4 8AA.**

761(A)

### North East Surrey College of Technology

Reigate Road

Ewell Surrey KT17 3DS

Principal: B. Haynes BSc DipEd CChem  
FRIC

## HEAD OF THE SCIENCES

The Governors wish to appoint a successor to the late W. C. A. Hards BSc CChem FRICS as Head of the Department of The Sciences with effect from September 1, 1979 or as soon as possible thereafter.

Salary: Burnham Head of Department (Grade IV) £7,941—£8,901 (under review).

Forms of application and further information about the post can be obtained from the Vice-Principal at the above address to whom applications should be returned by June 15, 1979.

739(A)



**SURREY  
COUNTY COUNCIL**

# Research Group Leader

## Pyrometallurgy Research Group

# Research Staff

### Research Group Leader

The Institute's Pyrometallurgy Research Group is based at the University of the Witwatersrand and is linked direct with the Process Development Division. The Group does a considerable proportion of the longer term research work and enjoys close interaction with the Division. Applicants should possess the following qualifications:

- (i) a research degree in an appropriate discipline.
- (ii) experience in high temperature extractive metallurgy.
- (iii) experience in the supervision of graduate students or graduate personnel, or both.
- (iv) the ability and perspective to handle a number of different projects that are being conducted concurrently.
- (v) sufficient industrial knowledge to interact and communicate with representatives of industry, and
- (vi) sufficient experience to warrant appointment at the level of Principal Scientist, or preferably, Chief Scientist.

### Research Staff

The Institute is looking for research staff to work on projects covering a wide field in minerals and metals. Applicants should be in possession of a research degree in chemical engineering, extractive metallurgy, instruments or electronic engineering, analytical chemistry, industrial or pure chemistry, material science, minerals processing, or a related field.

Post-graduate experience will be a recommendation, but is not essential. Benefits include a pension scheme, medical and a housing-subsidy scheme, 5-day working week, generous leave privileges (in addition to normal vacation leave, the Institute closes for the period between Christmas and New Year), flexitime, subsidised cafeteria facilities, sports facilities such as squash, tennis, swimming, snooker, etc. and an annual leave bonus.

There are possibilities for advanced studies at universities, with substantial bursaries from the Institute.

The Institute will pay the fares to South Africa of the applicant and his family, contribute to removal expenses, and grant a settlement allowance under certain conditions. Please apply giving full details of age, qualifications, experience, etc. to:

The Head: Personnel, National Institute for Metallurgy,  
Private Bag X3015, Randburg 2125, South Africa.

**National Institute for Metallurgy**

727(A)



## UNIVERSITY OF LIVERPOOL

### DEPARTMENT OF VETERINARY PATHOLOGY

### TECHNICIAN

to provide an efficient technical service in this department which is responsible for veterinary pathology, microbiology and immunology. Duties include supervising and training technical staff, ordering equipment and supplies and maintaining appropriate accounts and records. The successful candidate will also be responsible for technical services to ensure the smooth running of undergraduate teaching courses and advice to academic and other staff on specialised techniques and apparatus. Candidates must have had a minimum of 13 years laboratory experience, an APMIS or equivalent appropriate qualification.

Salary within the range £5,172 to £5,625 per annum.

Application forms may be obtained from The Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote Ref: RV/585/N. 695(A)

## UNIVERSITY OF SOUTHAMPTON

### DEPARTMENT OF BIOLOGY

Research Technician (biological sciences) required to support a research project aimed at evaluating the potential of fallow deer as farm animals. The post will involve full day to day running of feeding trials based at the Hampshire College of Agriculture, Sparsholt, Winchester.

Previous experience in the handling and management of large animals would be an advantage and applicants should hold a current driving licence. Minimum qualifications O.N.C./D.C. equivalent in science subjects (including biology) but graduates in appropriate disciplines are welcome to apply.

The appointment which is to be filled from August 1, 1979 will be for one year in the first instance with the possibility of renewal for a second year commencing with a salary of £2,757 per annum on grade 2B scale.

Applications giving date of birth, details of qualifications and experience and the names and addresses of two referees should be sent to Mr C. N. Saul, The University, Southampton SO9 5NH by June 1, 1979, quoting reference number 541/T/N. 741(A)

## DEPARTMENT OF GEOLOGY

### UNIVERSITY OF WINDSOR

has an opening at the rank of Assistant Professor, with specialisation in one or a combination of the following fields: Engineering Geology, Geotechnical Engineering, Structural Geology, Mining Geology or other applied disciplines. The position is a two-year appointment with review at the end of that period. It involves undergraduate- and graduate-level instruction, as well as conducting research programmes. Applicants should hold or anticipate a doctorate degree. Salary will be in the order of \$20,000 per year, depending on teaching, industrial and/or research experience. The position will commence as soon as possible, preferably September 1, 1979.

An application including a curriculum vitae and names of three referees should be sent to: Dr R. K. Jui, Chairman, Department of Geology, University of Windsor N9B 3P4. W104(A)

## QUEEN ELIZABETH COLLEGE

### Kensington

### (University of London)

### LECTURER IN FOOD SCIENCE

Applications are invited for the post of Lecturer in Food Science. Applicants will be expected to have an appropriate background in the Natural or Applied Sciences, preferably with research experience in an area of Food Science. The successful applicant will be required to teach some unit courses in Food Science to different years of the degree course and to undertake research. External contacts will be encouraged. Industrial or other relevant experience would be an advantage, and placing on the salary scale will reflect both qualifications and experience. Salary scale (under review) £3,909 to £7,754 per annum plus London Allowance of £502 per annum.

Apply to the College Secretary, Queen Elizabeth College, Campden Hill Road, London W8 7AH, from whom further particulars and an application form may be obtained. Closing date: June 8, 1979. 729(A)

## HARVARD MEDICAL SCHOOL

### NEUROBIOLOGIST

The Department of Neurobiology is seeking a new faculty member at the assistant-professor level to begin in 1979 or 1980. Applications from women and minority people are encouraged. Please send curriculum vitae and a brief outline of research plans, and have three letters of reference sent to: Search Committee, Department of Neurobiology, Harvard Medical School, 25 Shattuck Street, Boston MA 02115. W99(A)

# Research Officer

## Chemistry Section

NIBSC is an Institute concerned with the standardisation and control of biological substances used in medicine and is a WHO laboratory for biological standards. The work of the Institute is well supported by research.

Applications are invited for a Research Officer in the Chemistry Section. The post is in the laboratory concerned with polysaccharides and the application of methods of carbohydrate chemistry to analyse other substances such as antibiotics. Applicants should have or expect to gain this year a first degree in an appropriate subject.

**Salary, depending upon age and experience, on the scale £3,715—£5,259 p.a. (under review), including London Weighting, plus superannuation benefits.**

Please write or telephone for further details and an application form, quoting reference CH/161, to the Personnel Officer, NIBSC, Holly Hill, Hampstead, London NW3 6RB (Tel: 01-435 2232, ext. 200).

**NIBSC**

National Institute for Biological Standards and Control

ROYAL HOSPITAL for Sick Children and Queen Mother's Hospital, Glasgow G3 8SJ. Assistant Cytogenetic science graduate, required for West of Scotland Genetic Advisory Service, to work on the diagnosis of human chromosome aberrations and other disorders. Experience in cytogenetics and cell culture desirable but not essential. Further details may be obtained from the University Department of Medical Genetics (041-339-888; Ext. 7117). Applications, with full details of qualifications and experience and names of two referees, to Personnel Office, Royal Hospital for Sick Children, Yorkhill, Glasgow G3 8SJ 738(A)

# LOTHIAN HEALTH BOARD BASIC GRADE CYTOGENETICIST

Applications are invited from science graduates for the above post based at the Department of Pathology, Royal Hospital for Sick Children, Edinburgh. Experience in human cytogenetics desirable.

All types of cytogenetic investigation are undertaken including the pre-natal diagnosis of chromosome disorders, blood culture analysis, bone marrow investigation and the karyotyping of post-mortem and spontaneous abortion material. A broad training in cytogenetics would be afforded.

Arrangements to visit the department may be made by telephoning 331-667 1991, ext. 270.

Salary scale: £2,991 to £4,899 per annum with placement according to qualifications and experience.

Applications, which should be type-written, giving particulars of age, qualifications and previous experience, together with the names, addresses and telephone numbers of two referees should be lodged with the Secretary, 11 Drumshugh Gardens, Edinburgh EH3 7QQ by May 24, 1979. 686(A)

# THE UNIVERSITY OF MANCHESTER DEPARTMENT OF PHARMACY EXPERIMENTAL OFFICER

The person appointed will be responsible for the development and co-ordination of bioanalytical techniques in research dealing with drug metabolism and disposition. The techniques primarily involve the use of chromatography, spectroscopy and radioactivity. Applicants must be graduates in either chemistry, biochemistry, or a related science, with a minimum of two years relevant experience post degree. Initial salary in the range £3,384 to £5,604 per annum (under review), superannuation under U.S.S. Particulars and application forms (returnable by June 15) from the Registrar, The University, Manchester M13 9PL. Quote ref: 96/79/N. 692(A)

# ROYAL FREE HOSPITAL SCHOOL OF MEDICINE

(University of London)

DEPARTMENT OF  
PHARMACOLOGY

Applications are invited from

POSTDOCTORAL  
BIOCHEMISTS

For the post of Lecturer in Biochemical Pharmacology in the Department of Pharmacology, which is now housed in the Clinical Sciences Building, Royal Free Hospital, Hampstead. Some experience in muscle biochemistry desirable. Salary scale (under review) at present £3,909 to £7,754 plus £502 London Allowance and superannuation benefits. Applications (six copies) should be sent by June 1, 1979 to the Secretary, R.F.H.S.M., 8 Hunter Street, London WC1N 1BP, from whom further particulars may be obtained. 690(A)

# UNIVERSITY OF LIVERPOOL

DEPARTMENT OF ZOOLOGY

Applications are invited for the post of

SENIOR

RESEARCH ASSISTANT

in the Department of Zoology.

The project is on the adaptation of ion transport in erythrocytes of salinity-acclimated trout and involves isotope flux and cell culture techniques.

Initial salary £3,883, £4,133 or £4,382 per annum (under review).

Applications, together with the names of two referees, should be received not later than May 31, 1979, by The Registrar, The University, PO Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref. RV/584/N. 689(A)

# OVERSEAS DEVELOPMENT

KNOW-HOW: vital to developing countries

# Nutritionist

Bangladesh

Wanted for attachment to the Ministry of Relief and Rehabilitation to assist in the organisation and evaluation of various feeding schemes including a Food for Work programme and in association with a World Food Programme designed to mitigate the severity of malnutrition among mothers and children. Applicants should have a postgraduate qualification in nutrition and post qualification experience in a developing country.

Appointment 3 years. Salary (UK taxable) according to age and experience plus a variable tax free allowance currently in range £1,030—£2,640 according to marital status.

*The post is wholly financed by the British Government under Britain's programme of aid to the developing countries. In addition to basic salary and overseas allowances other benefits normally include paid leave, free family passages, children's education allowances and holiday visits, free accommodation and medical attention. Applicants should be citizens of the United Kingdom.*

*For full details and application form please apply, quoting ref. 319x stating post concerned, and giving details of age, qualifications and experience to:—*

723(A)



Appointments Officer,  
MINISTRY OF OVERSEAS DEVELOPMENT,  
Room 301, Eland House,  
Stag Place, London SW1E 5DH.

**HELPING NATIONS HELP THEMSELVES**

# UNIVERSITY OF LEICESTER DEPARTMENT OF ANATOMY RESEARCH ASSISTANT

Applications are invited for the post of Research Assistant on an M.R.C. funded project involving physiological and anatomical studies of the mammalian carotid body. The position is tenable for three years in the first instance and the salary will be on the grade 1B scale.

Interested persons should write to Dr D. J. Pallot, Department of Anatomy, University of Leicester, University Road, Leicester LE1 7RH, giving full personal details and the names of two referees. 733(A)

**INSTITUTE OF CANCER Research:** There is a vacancy for a TECHNICIAN to work in the Radiotherapy Research Laboratories at Sutton in a research programme on in vitro tests for the sensitivity of human tumour cells to cancer chemotherapeutic agents. The work involves specialised tissue culture techniques as well as some laboratory animal work with xenografted human tumours. Experience in pharmacology would be an advantage. The appointment will be on the Technician scale which starts at £3,261 per annum plus London Allowance of £354 per annum. Applications in duplicate with the names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place, London SW7 3NU, quoting ref. 301/B/62. 735(A)



# LEICESTER UNIVERSITY

# F. W. BENNETT CHAIR OF GEOLOGY

Applications are invited for the F. W. Bennett Chair of Geology formerly held by the late Professor P. C. Sylvester-Bradley. There is no restriction as to the special interests of applicants.

Further particulars from the Registrar to whom applications should be sent by June 15, 1979. Candidates in the U.K. should submit fifteen copies of their application (overseas candidates may submit one copy). 669(A)



LAMBETH is one of the foremost authorities in the country in the provision of comprehensive Environmental Health and Consumer Services. It has achieved this position through a combination of far sighted policies, adequate resources and highly qualified staff. The following vacancies now exist:

## RESEARCH OFFICERS(4)

Ref E34

Salary: £5,208 - £5,508 p.a. incl.

### Postdoctoral Research at the sharp end

Applicants should have a good record of academic achievement in preferably one or more of the following subject areas: Environmental Sciences; Political/Social Studies; Statistics; Economics; Marketing; Operational Research; Law.

The Directorate employs over 400 staff who are primarily engaged in providing operational services to the public. As a consequence, the Research facility is fundamental to policy and developments within the fields of Consumer Protection and Environmental Health. These posts offer an excellent opportunity for people with proven research capabilities to relate their skills to services designed to tackle the community's economic and environmental problems.

## INFORMATION AND PLANNING OFFICER

Ref E33

Salary: £4,680 - £6,003

The Directorate has just completed a major review of its support and administrative organisation. Now we are seeking an experienced analyst to spearhead the development of our management information and administrative systems in conjunction with the progressive application of word-processing, micro-filming and computerisation. In addition the Information and Planning Officer will contribute to the systems aspects of Committee Continuity, report preparation and corporate management particularly in respect of devising processes for target setting and effectiveness monitoring. The postholder should be able to familiarise him or herself with how the professional officers serve the public as well as meeting support requirements of senior management. You should be well qualified, both academically and professionally and we are interested in someone with a record of practical relevant achievements. A local government background is not essential.

If you would like an application form please contact the Personnel Section, Directorate of Environmental Health & Consumer Services, London Borough of Lambeth, Blue Star House, 234/244 Stockwell Road, London SW9 9SR. Tel: 01-274 7722 ext. 239. Closing date for completed applications 1st June 1979. These posts are open to both sexes and all races.

728(A)

# LAMBETH

## Senior Clinical Research Associate Guildford

Berk Pharmaceuticals, part of a major International Health Care Group, is engaged in the manufacture, marketing and distribution of a wide range of ethical drugs and is situated in a pleasant rural part of Surrey. Due to the continued expansion of our Medical Division we require a Senior C.R.A. who will play an important integral role in our clinical trials programme. You will take part in the research of compounds through phases 1, 2 and 3 of their development by designing protocols, actively participating in clinical studies and the assessment and presentation of results.

We are looking for either, someone who has a Ph.D. in the sciences or is working towards such a qualification, or a Senior Nurse with sound experience of Clinical Investigation work. Some knowledge of computing relating to the verification and processing of trial data would be an advantage but is not essential.

In addition to a competitive salary we offer 4 weeks annual leave, productivity bonus, pension scheme and other benefits.

If you are interested please telephone or write for an application form or send full C.V. to:—



Alan Ford, Personnel Department,  
Berk Pharmaceuticals Limited,  
Station Road, Shalford,  
Guildford, Surrey GU4 8HE.  
Tel: Guildford 71221 ext. 230.

720(A)

### UNIVERSITY OF NOTTINGHAM DEPARTMENT OF BOTANY

It is likely that one postgraduate research studentship will be available for work in one of the following areas:

- (1) Endocytosis and intracellular digestion in the ciliate *Tetrahymena*.
- (2) The cell cycle in green algae.
- (3) Nitrogen metabolism in *Rhodospirillum rubrum*.

- (4) Cytological studies on ferns.
- (5) Localisation of metabolic sites of action of herbicides in isolated mesophyll tissue.

- (6) Causal factors and physiological function of swollen lateral roots in the *Cyperaceae*.

- (7) Quantitative studies on plant cell ultrastructure.

If you are interested in research in one of these areas will you please write as soon as possible to Professor E. C. Cocking, Department of Botany, University of Nottingham, University Park, Nottingham NG7 2RD, indicating which area is of interest, giving a curriculum vitae and the names and addresses of two referees.

758(F)

### POSTDOCTORAL POSITION AVAILABLE

A postdoctoral position will be available in July, 1979 for appointees having either a Ph.D. in biochemistry or microbiology. Experience in enzymology desirable, though not critical. The project will be concerned with the regulation of carbohydrate metabolism during development in the slime mold, *Dictyostelium discoideum*.

Please send curriculum vitae and three references to Dr K. A. Killick, Boston Biomedical Research Institute, Department of Developmental Biology, 20 Staniford Street, Boston, Mass. 02114, U.S.A.

We are an "Equal Opportunity/Affirmative Action Employer."

W111(A)

### NATURE CONSERVANCY COUNCIL CHIEF WARDEN

Salary Range £4,809 to £5,475 (under review)

Applications are invited for the post of CHIEF WARDEN—SOUTH WALES, based in the N.C.C. Regional Office at Cardiff.

The successful applicant will be responsible to the Regional Officer for all management work in the National Nature Reserves in the South Wales Region. Duties will include the direction and supervision of the work of the two Reserve Wardens, of Estate Worker, and the temporary and Voluntary Wardens on Reserves, and the development and extension of the voluntary wardens system in the South Wales Region.

The Chief Warden will also be responsible for the formulation and forward working programmes, estimating and controlling the financial allocations for all estate work, advising on and implementing matters concerning the health and safety of staff and visitors on reserves, advising land owners and managers on land management matters, and contributing to and participating in the educational interpretative, survey and monitoring programmes of the Region.

Candidates should be over 26 years of age on June 15, 1979, physically fit and have a full current driving licence.

Further particulars and an application form can be obtained from:

The Nature Conservancy Council  
Recruitment Section  
P.O. Box 6  
George Street  
Huntingdon  
Cambs.

Tel. 0480 (Huntingdon) 56191  
Closing date for receipt of completed applications: June 1, 1979.

717(A)

# BEATSON INSTITUTE FOR CANCER RESEARCH SCIENTIST

(Geneticist or Biochemist)

There will be a vacancy from October 1, 1979 for a postdoctoral research scientist to join a team investigating the regulation of transcription of the globin genes in mammals. The work involves genetic manipulation, including the cloning of human genes, and previous experience in handling bacteriophage and/or nucleic acid enzymology is desirable. The Beatson Institute has category 2 and category 3 containment facilities and a core of staff with experience in genetic engineering. Several other projects using this technology are being pursued concurrently.

The appointment will be for three years in the first instance and will be at the appropriate point on the University of Glasgow's Research Assistant scales (which correspond to M.R.C. scales). M.R.C. terms of service apply. Applications should include a C.V. and names of referees. Address enquiries to:—

The Director,  
Beatson Institute for Cancer  
Research,  
Garscube Estate,  
Switchback Road,  
Beardsden,  
Glasgow G61 1BD.

726(A)

# NMR POSTDOCTORAL POSITION

Applications are invited for post-doctoral work on the NMR of systems of chemical and biological interest, under the direction of Professors H. S. Gutowsky and Eric Oldfield. Fourier transform spectrometers equipped with widebore superconducting magnets, operating at 360, 220 and 150 MHz, are available for use on the following projects: (1) Deuterium NMR of specifically  $^2\text{H}$ -labelled membranes. (2) Phosphorus-31 NMR studies of protein-lipid interaction in model and biological membrane systems. (3) High-resolution  $^{13}\text{C}$  NMR spectra of proteins in solution and in the crystalline solid state. (4) NMR studies of metal ion nuclei in solution and in the solid-state, with special emphasis on systems of biological importance (such as  $^{57}\text{Fe}$ ,  $^{23}\text{Na}$ ,  $^{39}\text{K}$ ,  $^{67}\text{Zn}$ ). Applications consisting of a curriculum vitae, list of publications and letters from three referees should be sent to: Professor H. S. Gutowsky, School of Chemical Sciences, University of Illinois at Urbana, Urbana, Illinois 61801, U.S.A. The University of Illinois is an Equal Opportunity/Affirmative Action Employer.

W110(A)

# UNIVERSITY OF SOUTHAMPTON

## MEDICAL ONCOLOGY UNIT

Applications are invited for the position of Postdoctoral Research Fellow in the above Unit. The person appointed will work on the isolation of cytoskeletal proteins and their interaction with the cell surface, in close cooperation with a group interested in cell adhesion and with others interested in clinical aspects of cancer research. Applicants should either be about to obtain or have recently obtained a Ph.D. in Biochemistry or some related discipline. Experience of protein biochemistry, membrane biochemistry or immunology would be advantageous. The appointment will be for one year in the first instance, renewable annually for at least three years.

Salary Range: £3,883 to £4,822 per annum (under review). U.S.S. benefits.

Applications giving date of birth, curriculum vitae and the names and addresses of two referees, should be sent to Mrs P. Vaughan-Smith, The University, Southampton SO9 5NH, not later than May 18, 1979. Please quote reference 1069/R/N.

598(A)

# LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

(University of London)

Keppel Street WC1 7HT

## DEPARTMENT OF MEDICAL MICROBIOLOGY A HISTOLOGY MEDICAL LABORATORY SCIENTIFIC OFFICER

is required to work for up to one year on histological research in immunopathology, hepatitis and mycotic infections: experience in histological techniques is essential. In addition, candidates should hold H.N.C./H.N.D./degree in a relevant subject. Salary is in the range £3,261 to £3,930 plus £354 London Weighting. Applications, consisting of full education and career details and naming two referees, should be sent to Secretary (A1) at the School.

742(A)

## DEPARTMENT OF PHYSICS UNIVERSITY OF CAMBRIDGE A RESEARCH ASSOCIATE

is required in the Cavendish Laboratory to assist with the development of scientific instrumentation used in Solid State and Surface Physics research and to be responsible for maintaining U.H.V. and computer-controlled electron-optical and cryogenics apparatus.

Experience and a high level of expertise in U.H.V. engineering design and in electronics is desirable. A degree or equivalent qualification is preferred although proven abilities in the above techniques are the prime consideration. The appointment is sponsored by the Royal Society for two years from September 1979 and there is a possibility of a tenured University post thereafter. Salary on a scale to £6,080 p.a. (under review) according to age and experience. Three copies of applications including a full c.v. and names of referees should be sent to Mr. J. Deakin, Secretary of the Department of Physics, Cavendish Laboratory, Madingley Road, Cambridge CB3 0HE before May 31.

710(A)

## THE UNIVERSITY OF MANCHESTER RESEARCH TECHNICIAN (GRADE 3)

### DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY, WITHINGTON HOSPITAL

Required to assist in the measurement of steroid hormone receptors in the female genital tract. Candidates should possess at least O.N.C. or equivalent and have experience in the measurement of steroid hormone receptors.

Suitable candidates may be considered for registration for higher qualifications.

Salary scale £2,931 to £3,336 per annum.

Applications including a curriculum vitae and the name and address of two referees (as to technical or academic ability) should be sent as soon as possible to Mrs B. Steele, Department of Obstetrics and Gynaecology, Research and Teaching Building, University Hospital of South Manchester, West Didsbury, Manchester M20 8LR not later than May 25, 1979.

732(A)

# CSIRO

# AUSTRALIA

## Assistant Chief Division of Animal Production Tropical Cattle Research Centre Rockhampton, Queensland

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees—2,400 of whom are research and professional scientists—located in Division and Sections throughout Australia.

**General:** Research in the Division of Animal Production is concerned with the solution of major problems in the animal industries, and in the provision of new and improved technologies that result in substantial gains in the efficiency of livestock production.

The Division has six broadly-based research programmes each of which is co-ordinated by a Programme Chairman. These programmes include work on nutrition, reproduction, genetics and animal breeding, skin and fleece biology, mineral requirements, and tropical animal production.

Current research at Rockhampton involves the genetic improvement of productivity in beef cattle for Northern Australia. Research is being expanded to complement this objective by developing new research initiatives in the fields of reproduction, nutrition and environmental adaptation.

### Duties:

1. To assist the Chief and to advise Programme Chairmen in the planning and development of research priorities directed towards the efficiency of livestock production in tropical and sub-tropical areas of Northern Australia. The research will, in the main, be directed towards studies on both the genetic and environmental factors which influence productivity. The Assistant Chief will initially be required to act as Programme Chairman of the Tropical Animal Production Programme.

2. To be responsible for the administration of the Rockhampton facilities which will include a major laboratory complex due for completion in 1980.

**Qualifications:** Applicants should have a substantial record of research achievement and an appreciation of problems in animal production in tropical and sub-tropical areas. In particular, applicants should have an established reputation of outstanding achievement in some field of animal production, e.g. genetics, environmental adaptation, nutrition of reproduction.

**Salary:** Senior Principal Research Scientist \$27,790 to \$30,530 p.a. or Chief Research Scientist Grade I \$31,407 p.a.

**Tenure:** This is an indefinite appointment which carries superannuation benefits subject to normal conditions. However, the role of Assistant Chief is offered for a term of three years. The situation will then be reviewed to determine whether the appointee should continue as Assistant Chief for a further period or as a senior research worker.

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 245/721 should reach:

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 9th June 1979.

Applications in U.S.A. and Canada should be sent to: The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, N.W., Washington, D.C., 20036, U.S.A.

756(A)

## UNIVERSITY OF EXETER DEPARTMENT OF GEOGRAPHY RESEARCH ASSISTANT

Applications are invited for a graduate research assistant to work with Dr E. Maltby in an investigation of differential trends in upland soil development resulting from reclamation of moorland for agriculture. Familiarity with techniques of chemical and physical analysis of soils and their description in the field would be an advantage.

The post which is supported by an N.E.R.C. research grant is tenable for two years from November 1, 1979. The initial salary is £3,384 plus U.S.S.

Applications and requests for further details should be addressed to Dr E. Maltby, Department of Geography, University of Exeter, Amory Building, Rennes Drive, Exeter EX4 4RJ as soon as possible.

684(A)

## OXFORD POLYTECHNIC DEPARTMENT OF BIOLOGY

### SENIOR LECTURER or LECTURER II IN PLANT PHYSIOLOGY

Applications are invited from suitably qualified candidates to take part in teaching and research in plant physiology as well as in the general teaching of ecology at honours degree and higher technician levels.

Salaries: Senior Lecturer £6,051 to £7,065 (bar) to £7,572. Lecturer II £4,101 to £6,558. Under review from April 1, 1979 (transfer from Lecturer II to Senior Lecturer is, subject to efficiency requirements, automatic).

Applications, including a curriculum vitae and the names of three referees, should be sent as soon as possible to the Head of Department of Biology, Oxford Polytechnic, Oxford OX3 0BP, from whom further particulars and application forms may be obtained.

722(A)

Centre for Applied Microbiology & Research,  
Porton Down, Salisbury, Wiltshire

## The Vaccine Research and Production Laboratory

has the following vacancies for suitably qualified scientists.

### 1. Vaccine Development

**A Microbiologist/Microbial Biochemist** to work in a small group concerned with the development of bacterial vaccines. The work will involve identification and separation of immunogenic components and production of trial batches for evaluation. Preference will be given to candidates who have up-to-date experience in microbial biochemistry and separation techniques.

### 2. Vaccine Assessment

**A Microbial Biochemist** to take a leading role in developing a programme concerned with methods for the assessment of potency and safety with particular reference to subunit vaccines. Preference will be given to candidates who have experience in immunology, biochemistry or pathogenicity.

### 3. Vaccine Production

**A Chief or Senior Medical Laboratory Scientific Officer** to participate in the development of a capability for vaccinia virus production, including quality control, storage and distribution of the vaccine. Research in viral vaccine development will be encouraged. Preference will be given to candidates with experience in virology and animal handling.

### 4. Vaccine Production

**A Medical Laboratory Scientific Officer** principally to supervise the tissue cell production unit but also to participate in the development of a vaccinia virus production capability. Preference will be given to candidates with experience in tissue culture and virology or animal handling.

Appointment for Posts 1 and 2 may be at either Senior Grade (£5,451 to £6,837) or Basic Grade Microbiologist (£2,991 to £4,899) depending upon qualifications and experience. A good honours degree or equivalent is essential and for a Senior Grade position a record of proven research ability.

Salaries on the Medical Laboratory Scientific Officer scale are: Chief (£5,472 to £6,192), Senior (£4,347 to £5,769), Basic Grade (£3,261 to £4,680), the assimilation point will depend upon qualifications and experience.

Further details of the posts can be obtained from Dr J. Melling, Director, Vaccine Research & Production Laboratory. (Tel. (0980) 610391).

*Applications stating date of birth, qualifications, experience and publications and naming three referees should be sent to Mrs M. Bushby, C.A.M.R., Porton Down, Salisbury, Wilts SP4 0JG by June 1, 1979.*

683(A)

**PH  
LS**

**Public Health Laboratory  
Service Board.**

#### UNIVERSITY COLLEGE DUBLIN PROFESSORSHIP OF GEOLOGY

The closing date for applications for the above appointment has been extended to **Thursday, May 31, 1979.**

Applicants who have not received acknowledgement of their applications and prospective applicants should telephone University College Dublin 693244, extensions 658, 657 or 416. 743(A)

#### ST HELIER HOSPITAL WRYTHE LANE CARSHALTON, SURREY SCIENTIFIC ASSISTANT

##### DERMATOLOGY DEPARTMENT

With postgraduate experience in either Biochemistry or Immunology to work on skin diseases including malignancy.

Apply to June 30 to Dr E. L. Rhodes at the above address. 759(A)

#### DIRECTOR DALTON RESEARCH CENTER UNIVERSITY OF MISSOURI COLUMBIA (Continuing Search)

This multidisciplinary facility for basic biological research, graduate and postgraduate training is supported principally from state funds, as well as from Federal agencies and foundations. A new building of 60,000 sq. ft. in Research Park, adjacent to the main campus, has excellent research laboratories with modern animal shop, computer, controlled environment, and other facilities.

A Director is sought with commitment to outstanding research who will develop a dynamic research unit in a life science area. The Director will be expected to bridge and complement Dalton Center activities with selected existing talents and resources on the UMC campus; examples are cardiovascular regulation, cellular and developmental biology, molecular genetics, immunobiology, neurosciences, and bioengineering.

This is intended as a "Center of Excellence" and the University is committed to providing a competitive Director's salary and other resources to insure success. The Director should have research management experience, including budgeting, recruiting, and generating research funds.

The Director will be tenured in an academic department of UMC. The University is a Land Grant school with a student enrolment of 23,000. On a single campus, it has a unique combination of life science-oriented Divisions (Medicine and other health related sciences, Agriculture, Veterinary Medicine, Engineering, and Biological Sciences). Particular research strengths may be found in departments of Biochemistry, Physiology, Biological Sciences, Bioengineering, Medicine, Microbiology, Pharmacology, Radiology, and Surgery. University resources include Reactor Research Center, Cancer Research Center, Health Services Research Center, Veterans Administration Hospital, University of Missouri Medical Center, and Eye Research Institute.

Women and members of minority groups are urged to apply. Resumes and other appropriate information should be sent to:

Search Committee for Director  
Dalton Research Center  
Research Park  
University of Missouri  
Columbia, Missouri 65211  
The University of Missouri is  
an Equal Opportunity Employer.  
W106(A)

#### UNIVERSITY OF LIVERPOOL DEPARTMENT OF BOTANY RESEARCH ASSISTANT

Applications are invited for the post of Research Assistant to assist in studies of numerical taxonomy and phage typing of streptomycetes (actinomycetes), in a joint research programme with the Department of Microbiology, University of Newcastle upon Tyne supported by S.R.C. Applicants should have or expect to obtain a degree in a biological science with experience in microbiology.

The Research Programme will last for one year starting in August 1979, and the salary is £3,384 per annum.

Applications, together with the names of two referees, should be received not later than May 25, 1979, by The Registrar, The University, PO Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref. RV/588/N. 697(A)

#### NATURE CONSERVANCY COUNCIL

SEABIRDS AT  
SEA RESEARCH  
SENIOR SCIENTIFIC  
OFFICER (1) AND HIGH  
SCIENTIFIC OFFICER (2)  
(3 year posts)

STARTING SALARY (at pre under review)

HIGHER SCIENTIFIC OFFICER

£4,101

SENIOR SCIENTIFIC OFFICER

£5,154

Salary on appointment may be in excess of these figures, depending on experience and qualification. Applications are invited for following four contract posts in Nature Conservancy Council's "birds at sea" Research Team. Posts are for a fixed period of 4 years only.

#### BACKGROUND

The seabirds at sea research programme is designed to investigate behaviour and distribution of seabirds offshore. Effort will be concentrated primarily in the North Sea and results will be used to refine N.C. response in relation to oil spill contingency planning.

#### THE POSTS

(a) **ORNITHOLOGIST** (Three posts) A sound knowledge of ornithology is required. A special interest in experience in seabird ecology desirable.

(b) **MARINE BIOLOGIST/OCEANOGRAPHER** (One post) A sound knowledge of marine biology/oceanography is required. A special interest in fish biology and some ornithological interests are desirable.

Experience in data handling will be an asset for both posts (a) and (b). One of the posts will be designated as team leader and will be graded S.S.O. The other members of team will be graded H.S.O.

The posts are based in Aberdeen. Candidates will be required to work offshore from boats or oil exploitation structures and carry out aerial surveys using light aircraft. The posts entail a good deal of travel and time away from base. A current driving licence is essential.

#### AGE AND QUALIFICATIONS

Candidates should have a first second class honours degree equivalent in a relevant scientific subject.

**HIGHER SCIENTIFIC OFFICER** Candidates should normally be under 30 years of age and have at least 3 years' relevant post-graduate experience.

**SENIOR SCIENTIFIC OFFICER** Candidates should normally be at least 25 and under 32 years of age and have at least four years' relevant post-graduate experience.

Application forms and further details are available from Miss Bull, Recruitment Section, Nature Conservancy Council, Godwin House, George Street, Huntingdon, Cambridgeshire PE18 6BU (Tel: 0480-56191, Ext. 2). Closing date for completed applications is June 1, 1979. 718(A)

#### TECHNICIAN/RESEARCH OFFICER

required in Tumour Immunology Unit at University College, WC, for a project investigating the role of macrophages in animal human immune systems. Work will involve a great deal of tissue culture, some animal work and other immunological techniques. H.N.C./degree essential.

Salary £3,615 to £5,256. For further information and application form write or telephone Miss S. M. Hurley at the Imperial Cancer Research Fund, Lincoln Inn Fields, London WC2 on 24 0200, ext. 305. 702(A)

**ASSISTANT PROFESSOR  
OF BIOCHEMISTRY  
LSU MEDICAL CENTER  
New Orleans, Louisiana**

The Department of Biochemistry, Louisiana State University Medical Centre, invites applications for 12 months tenure-track faculty positions. Candidates should have a strong background in biochemistry or molecular biology. In addition to the School of Graduate Studies, the Medical Center includes the Medical, Dental and Nursing Schools and the School of Allied Health Professions. Applicants should have postdoctoral experience and exhibit potential for developing a strong independent research program. Interest and excellence in teaching is also expected. Applicants should send a curriculum vitae, bibliography, a short summary of research goals, and have three letters of evaluation submitted to the following: Robert Roskoski, Jr., Head, Department of Biochemistry-LSU Medical Center—1542 Tulane Avenue, New Orleans, Louisiana 70112.

The Louisiana State University is an Affirmative Action/Equal Opportunity Employer. W108(A)

**UNIVERSITY OF  
SHEFFIELD  
LABORATORY  
SUPERINTENDENT  
(GRADE 8C)**

required for the Department of Geology from September 1, 1979, to be responsible for the operation of a complete technical service for teaching and research in an enlarged and modernised department. Candidates must have proven organising ability and considerable experience of science laboratories. Salary on scale £5,172 to £5,625 per annum (under review).

Please write to the Administrative Officer (Personnel), (Ref. S 1256/N), The University, Sheffield S10 2TN. 745(A)

**DEPARTMENT OF  
BIOPHYSICS  
King's College London  
POSTDOCTORAL  
RESEARCH ASSISTANT**

Postdoctoral position supported by the M.R.C. is available from June 1, 1979, to collaborate with Dr H. J. Gould on the molecular cloning and analysis of human immunoglobulin genes.

Starting date not later than September 1, 1979. Salary £3,883 to £6,555 p.a. plus London weighting.

Applications, with curriculum vitae and names of two referees, should be sent to Dr H. J. Gould, Department of Biophysics, King's College, 26-29 Drury Lane, London WC2B 5RL. 626(A)

**UNIVERSITY OF  
CAMBRIDGE  
DEPARTMENT OF GENETICS  
POSTDOCTORAL  
VIROLOGIST**

A position is available, from October 1, 1979, for 3 years, on a research project funded by the Science Research Council, for a postdoctoral virologist, preferably with experience in the molecular biology of viruses. Salary at age 27, £4,631, under review. Further details may be obtained from Dr M. Ashburner to whom applications, with the names of two referees, should be sent to reach him by June 30, 1979. Department of Genetics, Downing Street, Cambridge CB2 3EH. 707(A)

**PLANT MOLECULAR  
BIOLOGIST  
ZOECON CORPORATION**

Zoecon Corporation, a leader in innovative agricultural chemical research, is establishing a major research effort in higher plant molecular biology. Research directions will include the application of the techniques of molecular biology to crop improvement. Qualified scientists will join a research team working in a unique scientific environment. Zoecon offers an opportunity to develop and direct a research program, the intellectual stimulation of interaction with excellent colleagues, and the opportunity to contribute to the expansion of this exciting field. Individuals with expertise in plant genetics, biochemistry and physiology are encouraged to apply. The positions require a Ph.D. in a field of plant biology, preferably with post doc experience.

Zoecon is a division of Occidental Petroleum Corporation with substantial domestic and international operations. Corporate headquarters and corporate research are located in the Stanford Industrial Park, in close proximity to San Francisco. Submit detailed résumés in confidence to:

Kathy Moore  
Manager, Employee Relations  
Zoecon Corporation  
975 California Avenue  
Palo Alto, California 94304.  
W89(A)

**UNIVERSITY OF OXFORD  
POSTDOCTORAL  
RESEARCH ASSISTANT  
AND TECHNICIAN**

required for research into the regulation of branched chain 2-oxo acid dehydrogenase complex, branched chain amino acid catabolism and into disorders of branched-chain amino acid metabolism. The posts are for three years from a mutually convenient date in 1979 and are funded by the Medical Research Council. The postdoctoral research assistant should have experience in enzymology and protein chemistry and possess or expect a Ph.D. or equivalent degree. The technician should have a degree in biochemistry (or a related subject) or possess suitable laboratory experience. Starting salaries up to £4,382 (research assistant) and £3,930 (technician) on scales in operation on March 1, 1979.

Applications including the names of two referees to Professor P. J. Randle, Department of Clinical Biochemistry, Radcliffe Infirmary, Oxford OX2 6HE from whom further details may be obtained. 659(A)

**CHARING CROSS HOSPITAL  
MEDICAL SCHOOL  
(University of London)  
DEPARTMENT OF  
HAEMATOLOGY**

Non-clinical Lecturer required. Work of the appointee will be particularly concerned with surface membrane markers on lymphocytic and leukaemic cells, evaluation of immune complexes and assessment of immunoglobulin fractions. In addition the appointee will be encouraged to develop work on sub-cellular fractions. Teaching of immunology related to Haematology will be required. Post would suit a postdoctoral Fellow or equivalent.

Informal enquiries can be made to Professor G. D. Pegrum, Department of Haematology (01-748 2040, ext. 2703).

Salary on scale £3,883 to £7,754 (under review) per annum plus £502 London Weighting Allowance.

Applications, including the names and addresses of two referees, should be submitted to the School Secretary, Charing Cross Hospital Medical School, The Reynolds Building, St Dunstan's Road, London W6 8RP, by June 2, 1979. (Ref: 499/500.) 731(A)

**British Museum (Natural History),  
London.**

# Head of Freshwater Algae Section

The British Museum (Natural History) is an institution for taxonomic research and an educational focal point for the public. The main aim of the research is to produce definitive accounts of the world's animals, plants and minerals as well as the preservation of an extensive collection of fossils.

The successful candidate will supervise the work of junior staff, conduct research on the taxonomy of freshwater Chlorophyta and/or Rhodophyta and organise the Section's curatorial and advisory services.

Candidates, normally aged under 32, should have a good honours degree or equivalent in an appropriate discipline and at least two years' postgraduate experience. Experience in algal taxonomy is highly desirable.

Appointment as Senior Scientific Officer (£5,675 to £7,420) or Higher Scientific Officer (£4,620 to £5,970) according to qualifications and experience. *Salaries under review.* Promotion prospects. Non-contributory pension scheme.

For further details and an application form (to be returned by June 6, 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants. RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). *Please quote ref: SB/55/DK.*

740(A)

# Science Group CIVIL SERVICE

# CHAIRMAN DEPARTMENT OF MEDICINE

**THE UNIVERSITY OF CHICAGO  
PRITZKER SCHOOL OF MEDICINE**

Applications are invited for the position of Chairman of the Department of Medicine, The University of Chicago—Pritzker School of Medicine. Send letters of application and supporting information to:

**JARL E. DYRUD, M.D.,  
Associate Dean for Faculty**

**THE UNIVERSITY OF CHICAGO  
PRITZKER SCHOOL OF MEDICINE**

**950 E. 59th Street, Box 411  
Chicago, Illinois 60637**

An Affirmative Action/Equal Opportunity Employer

W112(A)



## UNIVERSITY OF NOTTINGHAM ENVIRONMENTAL PHYSICS AND PHYSIOLOGY

Applications are invited from graduates and post-graduates for posts tenable for three years on the following interdisciplinary projects supported by the Department of the Environment and the Natural Environment Research Council.

### RESPONSES OF WHEAT TO SULPHUR DIOXIDE (two posts)

Graduate research assistant—to develop a computer-controlled system for exposing areas of field crops to air pollution and to investigate turbulent fluctuations in pollutant concentrations. A suitably qualified applicant may register for a higher degree.

Postdoctoral research fellow—to study growth, development and yield of wheat in response to sulphur dioxide. Applicants should have a doctorate in an appropriate branch of biological science.

### FOG CAPTURE BY VEGETATION—A PATHWAY FOR POLLUTANT DEPOSITION

Postdoctoral research fellow—to measure fluxes of fog to a forest and to isolated trees and shrubs and to determine pollutant concentrations in the fog droplets. Part of the field work will be Northern England and Central Scotland. Applicants should preferably have a doctorate in physics, meteorology or related branches of physical science.

### THE HEAT BALANCE OF WET VEGETATION

Ph.D. Research studentship—to make micrometeorological studies of factors determining the duration of leaf wetness and to develop computer simulation models. A good honours degree in physics or environmental science is desirable.

#### Salaries

Postdoctoral fellow: on the scale £4,333 to £4,910.

Graduate assistant: on the scale £3,775 to £4,333.

Research student: Research Council rates plus fees and allowances.

For further details of all projects write to Dr M. H. Unsworth, Department of Physiology and Environmental Studies, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough LE12 5RD. 693(A)

## DIRECTOR INSTITUTE OF MARINE SCIENCE UNIVERSITY OF ALASKA

The University of Alaska, Fairbanks, invites applications for the position of Director, Institute of Marine Science and Division of Marine Sciences. Qualifications sought are a doctoral degree, leadership in interdisciplinary research management, experience in dealing with funding sources, and a knowledge of budgetary operations. A distinguished research record is expected. Experience in teaching and the supervision of thesis projects of graduate students is desirable. Marine Sciences at the University have a full-time faculty of 23 and a graduate student enrolment of about 40.

Applications or nominations with biographical and other pertinent information should be received before July 1, 1977, by the chairman of the search committee:

Dr Gunter E. Weller  
Geophysical Institute  
University of Alaska  
Fairbanks, Alaska 99701 U.S.A.

The University of Alaska offers equal educational and employment opportunities. W113(A)

## FACULTY POSITIONS PARASITOLOGY

The Department of Pathobiology, University of Pennsylvania School of Veterinary Medicine is inviting applications for faculty positions in the Laboratory of Parasitology. Applicants are expected to have a D.V.M. and/or Ph.D. degree and have a demonstrated ability and interest in research and teaching in parasitology. Major research interests in the laboratory at present are in the areas of immunoparasitology and ecological parasitology. Faculty rank and salary open. Candidates are requested to send resumes and names of references to Dr W. C. Wilcox, Chairman of the Search Committee, Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pa. 19174.

An equal opportunity, affirmative action employer. W109(A)

## UNIVERSITY OF NEWCASTLE UPON TYNE THE ENERGY CENTRE

Postgrad. or Postdoc. from suitable discipline (Science, Engineering, Statistics, or possibly Economics or Social Sciences) with interest in energy matters, for research project on Risks of Conventional Electricity Production.

Initially one year contract; salary according to qualifications and experience.

Write, enclosing curriculum vitae, to Professor G. R. Bainbridge, Director, The Energy Centre, University of Newcastle upon Tyne, 37 Jesmond Road, Newcastle upon Tyne NE2 4EU. 709(A)

UNIVERSITY COLLEGE Hospital. Scientific Officer (two posts), Applications are invited from suitably qualified graduates to work in the Haematology Research Department on the antenatal diagnosis of thalassaemia. Salary on the scale of £3,345 to £5,253 dependent upon qualifications and experience. Further information from Professor Huehns, Clinical Haematology Department, University College Hospital, Medical School, University Street, WC1. Application forms from Personnel Department, University College Hospital, Gower Street, WC1. Closing date: 10 days after appearance of this advertisement. 737(A)

## UNIVERSITY OF LIVERPOOL DEPARTMENT OF GEOLOGY

Applications are invited for the post of

### DEMONSTRATOR/ SENIOR DEMONSTRATOR

in the Department of Geology. Applicants should have an interest in some aspect of structural geology and experience of field studies is essential. Teaching duties will be concerned mainly with structural courses.

The post is tenable from October 1, 1979, for one year in the first instance, renewable yearly though not normally more than twice.

Initial salary on the scale £3,384 to £4,882 per annum (under review).

Applications, together with the names of three referees, should be received not later than May 25, 1979, by the Registrar, The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/589/N. 696(A)

## QUEENSLAND INSTITUTE OF TECHNOLOGY SENIOR LECTURER— HUMAN PHYSIOLOGY

Applications are invited from suitably qualified medical graduates for this newly established full-time tenured post. The appointee should have been involved in tertiary teaching and would be required to exercise leadership in the teaching of human physiology. The pursuit of a research interest would be encouraged and professional experience programmes are available. A generous superannuation scheme operates.

Salary will be within the range A\$21,180 to A\$24,687 per annum.

Full details of the post and the Institute are available from the Agent General for Queensland, 392 The Strand, London WC2R 0LZ or telephone (01) 836 3224.

Applications quoting V.51/79 and containing the names of three referees should reach the Personnel Officer, Q.I.T., George Street, Brisbane 4000 Australia by July 27, 1979. W103(A)

## ROTHAMSTED EXPERIMENTAL STATION HARPENDEN, HERTS. AL5 2JQ

Applications are invited from suitably qualified scientists for the post of Head of the Nematology Department which will become vacant in late 1979 on the retirement of Dr F. G. W. Jones.

The Nematology Department has about 40 scientific staff and receives many visiting workers.

The Department studies nematodes known or suspected to be plant parasites, and the research programme includes both fundamental and applied investigations. The Head of Department will be expected to encourage collaboration with other relevant disciplines at Rothamsted, and with other sections of the agricultural industry as appropriate.

Appointment in the grade of S.P.S.O., £10,043 by two increments to a maximum of £11,300 (under review). Non-contributory superannuation.

Apply in writing to the Secretary giving names and addresses of two referees and quoting Ref. 389 by June 8, 1979. Further details on request. 712(A)

RESEARCH GROUP requires laboratory scientific officer to assist genetic and cell biology work involving tissue culture. Candidates with relevant experience preferred. Applications should be sent to the Administrative Assistant, Paediatric Research Unit, The Prince Philip Research Laboratories, Guy's Hospital Medical School, London Bridge SE1 9RT. 736(A)

## ASSISTANTSHIPS

### UNIVERSITY OF DUNDEE DEPARTMENT OF CHEMISTRY

Applications are invited for a  
**POSTDOCTORAL  
RESEARCH  
ASSISTANTSHIP**

for research with Dr J. A. Miller on applications of organophosphorus chemistry to problems in organic synthetic methods and in natural product synthesis.

The preferred starting date is October 1, 1979 but earlier or later dates are negotiable. The appointment, which is financed from an S.R.C. grant is available for one year in the first instance but renewal for a second year is likely. Initial salary dependent on age, qualifications and experience but within the range £3,883 to £4,365 (under review).

Applications, with curriculum vitae and the names of two referees and quoting Ref: EST/49/79J should be sent as soon as possible (and not later than May 21, 1979) to The Secretary, The University, Dundee DD1 4HN. 706(P)

## THE QUEEN'S UNIVERSITY OF BELFAST A POSTDOCTORAL RESEARCH ASSISTANT

Department of Pure and  
Applied Physics

Applications are invited from men or women for a postdoctoral research assistantship supported by the Science Research Council in connection with an experimental study of lipid monolayers (with particular reference to their phase transitions) using laser light scattering techniques. The appointment is tenable for up to three years and initial salary will be in the range £3,885 to £4,383 (under review). Experience in computing is required; relevant experience in either lipid monolayers or photon correlation would be advantageous.

Applications with curriculum vitae and the names and addresses of two referees to the Personnel Officer, The Queen's University of Belfast BT7 INN, Northern Ireland. Closing date May 28, 1979. 687(P)

**UNIVERSITY OF CAMBRIDGE**  
Department of Botany. Postdoctoral Research Assistant. Applications are invited for a postdoctoral research assistantship to work on the biochemistry of the chloride-pump of sea avenger. The post, which is supported by the Science Research Council, is for a period of three years from October 1, 1979. The salary will be within the range 1A, starting at £3,883 to £4,382 (under review). Applications with curriculum vitae and the names and addresses of two referees should be sent as soon as possible to Dr D. E. Hanke, Botany School, Downing Street, Cambridge CB2 3EA, from whom further particulars may be obtained.

750(P)

**OXFORD UNIVERSITY**  
**INORGANIC CHEMISTRY**  
**LABORATORY**  
**POSTDOCTORAL RESEARCH**  
**ASSISTANTS: OPTICAL AND**  
**MAGNETIC PROPERTIES OF**  
**INORGANIC SOLIDS**

Applications are invited from chemists or solid-state physicists for up to three Postdoctoral Research Assistantships to work on preparation and crystal growing and on the magnetic, magneto-optical and optical properties of inorganic and metal-organic solids. Experience of either crystal growing, magnetic measurements, magneto-optics or laser spectroscopy would be an advantage. Tenable up to three years from October 1, 1979. Salary on the Science Research Council Scale 1A (£3,883 to £6,555) plus U.S.S. Applications should be sent to Dr P. Day or Dr R. G. Jennings, Inorganic Chemistry Laboratory, South Parks Road, Oxford OX1 3QR, from whom further details may be obtained.

744(A)

**UNIVERSITY OF OXFORD**  
**LABORATORY OF MOLECULAR**  
**BIOPHYSICS**  
**M.R.C.**  
**POSTDOCTORAL**  
**RESEARCH ASSISTANTSHIP**  
**IN BIOCHEMISTRY**

A post-doctoral position is available or a biochemist to work in collaboration with crystallographers on the relationship between the structure and function of glycogen phosphorylase *b*. The structural studies have reached a stage where it is possible to make definite proposals about mechanism. The biochemist would be involved in the design of experiments to test these proposals and in initiating his or her own programme of research on phosphorylase and related enzymes.

The appointment is for 3 years. Salary is on scale Grade 1A in the range £4,365 to £5,506, according to age. Applications with the names of two referees should be sent to Dr. N. Johnson, Laboratory of Molecular Biophysics, South Parks Road, Oxford OX1 3PS, from whom further information may be obtained. 711(P)

**STUDENTSHIPS**

**AGRICULTURAL**  
**RESEARCH COUNCIL**  
**STUDENTSHIP**

Applications are invited for a Studentship to work in the Protein Section of the Meat Research Institute.

The project is to investigate the turnover and remodelling of the connective tissues in relation to growth and development.

This will consist of radioactive incorporation studies together with autoradiography, the chemical analysis of connective tissue components and an examination of the cellular mechanisms for resorption of tissue.

The project is expected to form the basis for a Ph.D. thesis in the University of Bristol and the appointment will commence in October, 1979. Candidates should possess a First or Upper Second Class degree in Biochemistry.

Further particulars of the award and allowances together with Application Forms may be obtained from the Personnel Officer, Agricultural Research Council, Meat Research Institute, Langford, Bristol BS18 7DY.

747(F)

**UNIVERSITY OF LEICESTER**  
**DEPARTMENT OF**  
**MICROBIOLOGY,**  
**THE MEDICAL SCHOOL**  
**M.R.C. RESEARCH**  
**STUDENTSHIP**

Applications are invited from good honours graduates in a biological science and from students who expect to graduate in 1979 for an M.R.C. Research Studentship to investigate dimorphism and pathogenicity in *Candida albicans*. The work will involve biochemical analyses, electron microscopy and animal experimentation.

The studentship is tenable for three years, subject to satisfactory progress, and the successful candidate will be registered for the degree of Pr.D.

Applicants should write by May 25 to Dr F. C. Odds, Department of Microbiology, University of Leicester, Leicester LB1 7RH, enclosing a curriculum vitae and the names and addresses of two referees. 725(F)

**UNIVERSITY OF BRISTOL**  
**DEPARTMENT OF ZOOLOGY**  
Applications are invited for an  
**M.R.C.**

**RESEARCH STUDENTSHIP**

in Neurobiology to join a project with Dr Alan Roberts on central nervous mechanisms underlying swimming movements of amphibian embryos. Intracellular recording, neuroanatomy and digital computer modelling of neural networks will be involved.

Applicants should be interested in one of these approaches and have (or expect) a good degree in Science or Engineering. The post will be for three years from October 1979.

Applications should be sent to Suzy Oakes, Department of Zoology, University of Bristol, Woodland Road, Bristol BS8 1UG as soon as possible, and should include the names and addresses of two referees and a curriculum vitae. 699(F)

**UNIVERSITY OF**  
**MANCHESTER**  
**DEPARTMENT OF**  
**MEDICAL BIOPHYSICS**  
**RESEARCH STUDENTSHIP**  
**IN BIOPHYSICS**

Applications are invited from students having, or expecting, a good honours degree in science for an M.R.C. Research Studentship to undertake postgraduate research training in Biophysics, commencing October 1979. The successful candidate will be offered a choice of research projects and will be registered with the University for a higher degree.

Further details from Dr J. A. Chapman, Reader in Biophysics in the Department of Medical Biophysics, University of Manchester, Stoford Building, Manchester M13 9PT, to whom a detailed application, including names and addresses of two referees, should be sent as soon as possible. 721(F)

**THE POLYTECHNIC**  
**WOLVERHAMPTON**  
**DEPARTMENT OF**  
**BIOLOGICAL SCIENCES**  
**N.E.R.C. C.A.S.E.**  
**STUDENTSHIP**

To investigate aspects of decomposition of organic matter in soil in order to devise a standardised test to be used as an index of the effects of pesticides on decomposition.

Supervisors: Dr D. J. L. Harding and G. Ayers (Wolverhampton); Dr C. A. Edwards (Rothamsted Experimental Station, Harpenden).

Applicants should have or expect to obtain a good honours degree in biology or ecology, preferably with experience in microbiology.

The student will be registered with C.N.A.A. for M.Phil./Phd. Further details and application forms are available from "Personnel" The Polytechnic, Wolverhampton WV1 1LY. 754(F)

**THE UNIVERSITY**  
**OF MANCHESTER**  
**DEPARTMENT OF PHYSIOLOGY**  
**RESEARCH STUDENTSHIPS**

Applications are invited for three postgraduate research studentships (M.R.C. and S.R.C. tenable in the Department of Physiology from October 1, 1979. The value of the awards will be according to Research Council Scales. These awards are for research into: secretion of electrolytes and enzymes by exocrine glands; renal function in pregnancy; human circadian rhythms; cardiovascular reflexes; central neurotransmitters; cerebellar neurophysiology; physiology of dystrophic muscle. Eligible candidates should hold or expect to obtain a good Honours degree in physiology, pharmacology, biochemistry or other related subjects. Applications, together with the names and addresses of two referees should be sent as soon as possible to Dr A. C. Wareham, Department of Physiology, The Medical School, University of Manchester, Manchester M13 9PT. Telephone: 061-273-8241. 691(F)

**UNIVERSITY OF**  
**NEWCASTLE UPON TYNE**  
**DEPARTMENT OF**  
**DERMATOLOGY**

**Postgraduate Research**  
**Studentship**

Applications are invited from honours graduates, or prospective honours graduates, for a Junior Research Associateship which is available for up to three years in the Department of Dermatology for research into the action of pigmentary peptides in the skin. The research methods will include bioassay in conjunction with radioimmunoassay and chromatography. The successful candidate will be required to register for a higher degree and will be awarded maintenance allowances equivalent to those of M.R.C./S.R.C. studentships.

Applications giving a brief curriculum vitae and the names of two referees should be submitted by May 25, 1979 to Professor S. Shuster, Department of Dermatology, Royal Victoria Infirmary, Newcastle upon Tyne. 734(F)

**UNIVERSITY OF**  
**NEWCASTLE UPON TYNE**  
**Muscular Dystrophy Group**  
**Research Studentship**

Applications are invited from graduates in physiology, pharmacology, zoology or other appropriate biological sciences for a research studentship. The successful applicant will work on aspects of neuromuscular transmission in mice suffering from inherited diseases of the peripheral nervous system, and will need to adopt a multidisciplinary approach involving both physiological and morphological studies.

The studentship (value £1,610 per annum) is tenable for three years and will be held at the Muscular Dystrophy Group Laboratories, Newcastle upon Tyne.

Applicants should contact Dr J. B. HARRIS, Muscular Dystrophy Group Laboratories, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne NE4 6BE for further details. Applications should consist of a curriculum vitae, a statement of academic interests and the names of two referees and should be sent directly to Dr J. B. HARRIS, at the above address by June 8, 1979. 752(F)

**UNIVERSITY OF**  
**ABERDEEN**  
**DEPARTMENT OF**  
**MICROBIOLOGY**

The Department has a number of  
**RESEARCH STUDENTSHIPS**

available from October 1979. These will be for research in the areas of:—kinetics and genetics of bacterial transport systems; microbial interactions in soil nitrification; wall synthesis and morphogenesis in pathogenic fungi; origins and function of chitinase in fish; physiology and ecology of sulphate reducing bacteria in relation to North Sea oil industry.

Enquiries and applications from suitably qualified graduates or final year students in microbiology and related disciplines, should be addressed to Head, Department of Microbiology, University of Aberdeen, Marischal College, Aberdeen AB9 1AS. 704(F)

**COURSES**

**EMBO COURSE**  
**ELECTRON MICROSCOPY**  
**OF NUCLEIC ACIDS**

An advanced practical course on electron microscopy of nucleic acids will be held at the University of Ulm, from October 8 to 13, 1979.

The practical work includes: Heteroduplex analysis, Partial denaturation mapping of DNA, Secondary structure analysis of RNA, Cross-link reactions in nucleic acids, Molecular darkfield/brightfield electron microscopy. Instructors are competent researchers in the field.

Participants will be limited to 12 and selected from applicants having some experience with nucleic acid studies and/or electron microscope techniques. Limited funds will be available to cover travel expenses and/or a few stipends for accommodation. Applications should be sent to the Organisers, not later than August 15, 1979: Dr G. Klotz, Dr A. K. Kleinschmidt, Department of Microbiology, University of Ulm, Postfach 4066, D-7900 Ulm (Germany). W98(D)

**AWARDS**

**VETERANS ADMINISTRATION** announces the Second Round—Alcoholism Program. Emphasis on basic research to address etiology of alcoholism. Three year awards, up to \$100,000 per year. Applications accepted from VA and non-VA scientists, Ph.D.'s and M.D.'s. Research to be conducted in a VA Medical Center. Applications procedure: Letter of intent to apply due June 1, 1979. Ten page application due July 15, 1979. Decision on awards funding before January, 1980. Application should include the following: (1) Proposed investigation; (2) Curriculum vitae; (3) Two letters of recommendation. For further information contact: Dr Marcus Rothschild, VA Medical Center, New York, New York 10010 (212) 886-7500 X405. Dr Robert Allen (151F), VA Central Office, Washington, D.C. 20420. (202) 389 2124. W100(N)

**FELLOWSHIPS**

**UNIVERSITY OF WARWICK**  
**POSTDOCTORAL**  
**RESEARCH FELLOWSHIP**  
**IN INORGANIC CHEMISTRY**

Applications are invited for an S.R.C. Research Fellowship in the Department of Chemistry and Molecular Sciences for work on preparative, structural and catalytic properties of co-ordination and organometallic compounds of titanium, zirconium and hafnium in their lower oxidation states. The work is in conjunction with Professor M. G. H. Wallbridge and Dr G. R. Willey, and is for one year in the first instance with the possibility of renewal. Salary on the Research Range 1A scale, £3,883 to £6,555 (under review). Applications forms and further particulars may be obtained from the Academic Registrar, University of Warwick, Coventry CV4 7AL. UK. Please Quote Ref. 39/3A/79. 692(F)

### UNIVERSITY OF STRATHCLYDE

#### Applications are invited for a POSTDOCTORAL FELLOWSHIP IN ANALYTICAL CHEMISTRY

in the DEPARTMENT OF PURE AND APPLIED CHEMISTRY, tenable for two years, to work on the development and application of analytical techniques for the study of trace element metabolism during intravenous nutrition and in chronic haemodialysis with particular reference to selenium and chromium. Preference may be given to applicants with experience in atomic spectroscopy, as the principal techniques to be used will be flame atomic fluorescence and carbon furnace atomic absorption and emission spectrometry but consideration will be given to all candidates with a strong interest in the application of analytical techniques to clinical biochemistry. The post is sponsored by the Scottish Home and Health Department and will be jointly supervised by Dr J. M. Ottaway of the University of Strathclyde and Dr G. S. Fell of the Royal Infirmary, Glasgow.

The applicant will join an active research team involved in a wide range of trace metal metabolic studies in human medicine.

Commencing salary up to £5,637 per annum, under review. Range 1A of the national salary structure for research and analogous staff. Placing according to qualifications and experience. Superannuation benefit.

Further particulars (quoting R15/79) may be obtained from Dr J. M. Ottaway, Department of Pure and Applied Chemistry, University of Strathclyde, Cathedral Street, Glasgow G1 1XL, to whom applications should be sent as soon as possible.

694(E)

### THE ROYAL SOCIETY THE SMITH & NEPHEW FOUNDATION SENIOR

#### RESEARCH FELLOWSHIP

Applications are invited by the Council of the Royal Society for The Royal Society Smith & Nephew Foundation Senior Research Fellowship, established by means of a five-year grant from the Smith & Nephew Foundation for research in organic chemistry or biochemistry. The appointment will be tenable in any appropriate university department or research institution in the United Kingdom approved by the Council of the Royal Society. Candidates, who must be citizens of the United Kingdom or the Commonwealth, must supply the usual personal details and give two referees. Testimonials will not be considered. The subject of the proposed research, and where it would be done, together with the name of the head of the department, whose consent should first be obtained, must be stated.

The appointment which will be subject to the Society's general regulations governing research appointments, will be tenable for five years from October 1, 1979 (or another date to be arranged). Applicants should be under 40 on October 1, 1979. The present stipend scale is £8,031 by £252 to £8,787 by £249 to £9,036 per annum, and the point of entry will be determined by Council. Superannuation benefits will be provided. Some provision for research expenses will be available.

Applications should be made on forms to be obtained from the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, and returned by June 4, 1979.

685(E)

### IMPERIAL CANCER RESEARCH FUND RESEARCH FELLOWSHIP

A Postdoctoral Biochemist or Molecular biologist with experience in DNA sequencing techniques is required to assist in cloning and to sequence biologically significant regions of higher eukaryotic DNA.

The appointment will be for three years. Salary range £5,384 to £6,582 inc. L.A.

For further information telephone Dr A. M. Fried (01-242 0200 ext. 297). Applications with curriculum vitae and the names of two referees should be sent to the Secretary Imperial Cancer Research Fund, Lincoln's Inn Fields, London, W.C.2, quoting Reference 235/79 by June 4, 1979.

703(E)

### ST. PATRICK'S COLLEGE MAYNOOTH

#### DEPARTMENT OF CHEMISTRY

#### DUBLIN UNIVERSITY TRINITY COLLEGE

#### DEPARTMENT OF PURE AND ALLIED PHYSICS

#### POSTDOCTORAL RESEARCH FELLOWSHIP IN SURFACE SCIENCE

Applications are invited for the above post, funded by the National Board for Science and Technology, available immediately, tenable for a period of one year and renewable for a second, and final, year, at a salary of £4,190 per annum.

The Research Fellow will collaborate with Professor C. M. Quinn and Dr J. F. McGillo on experimental and theoretical studies of electron scattering from clean and adsorbate-covered single crystal surfaces.

Applicants should in the first instance, telephone the Staff Office, Trinity College, on Dublin 772941, Ext. 1678/1775.

W107(E)

### UNIVERSITY COLLEGE DUBLIN

#### DEPARTMENT OF BIOCHEMISTRY

#### Applications are invited for a POSTDOCTORAL

#### RESEARCH FELLOWSHIP

in the Department of Biochemistry, Belfield, Dublin 4. The successful applicant will help to develop a project (sponsored by Nordic Cold Storage Ltd.) investigating enzymatic changes occurring during long term storage of frozen meat products, and will therefore be expected to have adequate postgraduate experience in food science or in related fields. Appointment will be made on an annual basis for up to three years on a salary scale of £3,883 to £4,382 per annum.

Applications, with curriculum vitae and the names of two referees, should be sent, as soon as possible, but not later than May 31, 1979 to one of the following addresses: The Secretary, Department of Biochemistry, University College, Belfield, Dublin 4 or Mr W. J. A. Fearn, 1, The Sanctuary, Westminster SW1P 3JT, London, England.

714(E)

### UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL DEPARTMENT OF BIOCHEMISTRY POSTDOCTORAL FELLOWSHIP

Applications are invited for an M.R.C. funded post to investigate the properties of the muscarinic acetylcholine receptor on neuroblastoma cells in tissue culture; particular emphasis is to be made on the role of cyclic nucleotides in receptor function.

The post is for one year renewable for a further year at a starting salary of £3,883 per annum and the successful applicant will start on September 1, 1979 (or later as arranged).

Applications, including a curriculum vitae and the names of two referees, should be sent as soon as possible to Dr P. G. Strange, Department of Biochemistry, Medical School, Queen's Medical Centre, Nottingham NG7 2UH.

757(E)

### DALHOUSIE UNIVERSITY DEPARTMENT OF BIOCHEMISTRY

#### POSTDOCTORAL FELLOWSHIP

A position is available immediately to do cell cycle research with yeast. A background in biochemistry, molecular biology, cell biology, or microbiology desirable. Send Curriculum Vitae and names of three references to Dr R. A. Singer, Department of Biochemistry, Dalhousie University, Halifax, Nova Scotia, CANADA.

W105(E)

### CONFERENCES

#### CALL FOR ABSTRACTS BIOPHYSICAL DISCUSSIONS Second Discussion—May 1980

#### PROTEINS AND NUCLEOPROTEINS: STRUCTURE, DYNAMICS AND ASSEMBLY

The Biophysical Society will hold its 2nd Biophysical Discussion at Airlie House, Airlie, Virginia (near Washington, D.C.) on May 18-21, 1980. This Discussion will consider recent advances in understanding the principles of macromolecular structure and dynamics. Sessions will be devoted to: elucidation of structure by diffraction and spectroscopic methods; nature of forces stabilizing macromolecular structure; fluctuations in macromolecular structures; and mechanisms of folding and assembly. Experimental systems to be discussed include proteins, viruses, and organelles involving protein-nucleic acid interactions.

Prior to the meeting, all participants will receive a study book containing the full Discussion papers and poster abstracts. There will be no formal presentation of papers at the meeting, only a five-minute reminder followed by open discussion. A \$175 fee will cover registration, three days' room and board, the study book, and the final proceedings volume.

Papers submitted for the Organizing Committee's consideration are due as follows:

July 16, 1979—Preliminary abstracts (<300 words, to be reviewed and selected by August 1st)

December 1, 1979—Complete manuscripts (to be refereed and selected by mid-January)

The full edited proceedings of ca. 500 pp. will be published in hardback by Rockefeller University Press (\$20 prepublication, \$30 after October 1980). Identical text will be received by *Biophysical Journal* subscribers (1980 subscription, 12 issues, \$150). For these publications, remit to Order Service, Rockefeller University Press, P.O. Box 5108, New York, N.Y. 10249, USA.

For further information contact Valerie Parsegian, Executive Secretary, Biophysical Discussions, P.O. Box 30239, Bethesda, Maryland 20014, USA. Phone (202) 362-8184.

W93(C)



We have much pleasure  
announcing that the  
Queen's Award for  
Technological  
Achievement has been  
conferred on the  
Company.

The award has been made  
for work, in collaboration  
with the Royal Signals  
Radar Establishment and  
the Department of  
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University on Liquid  
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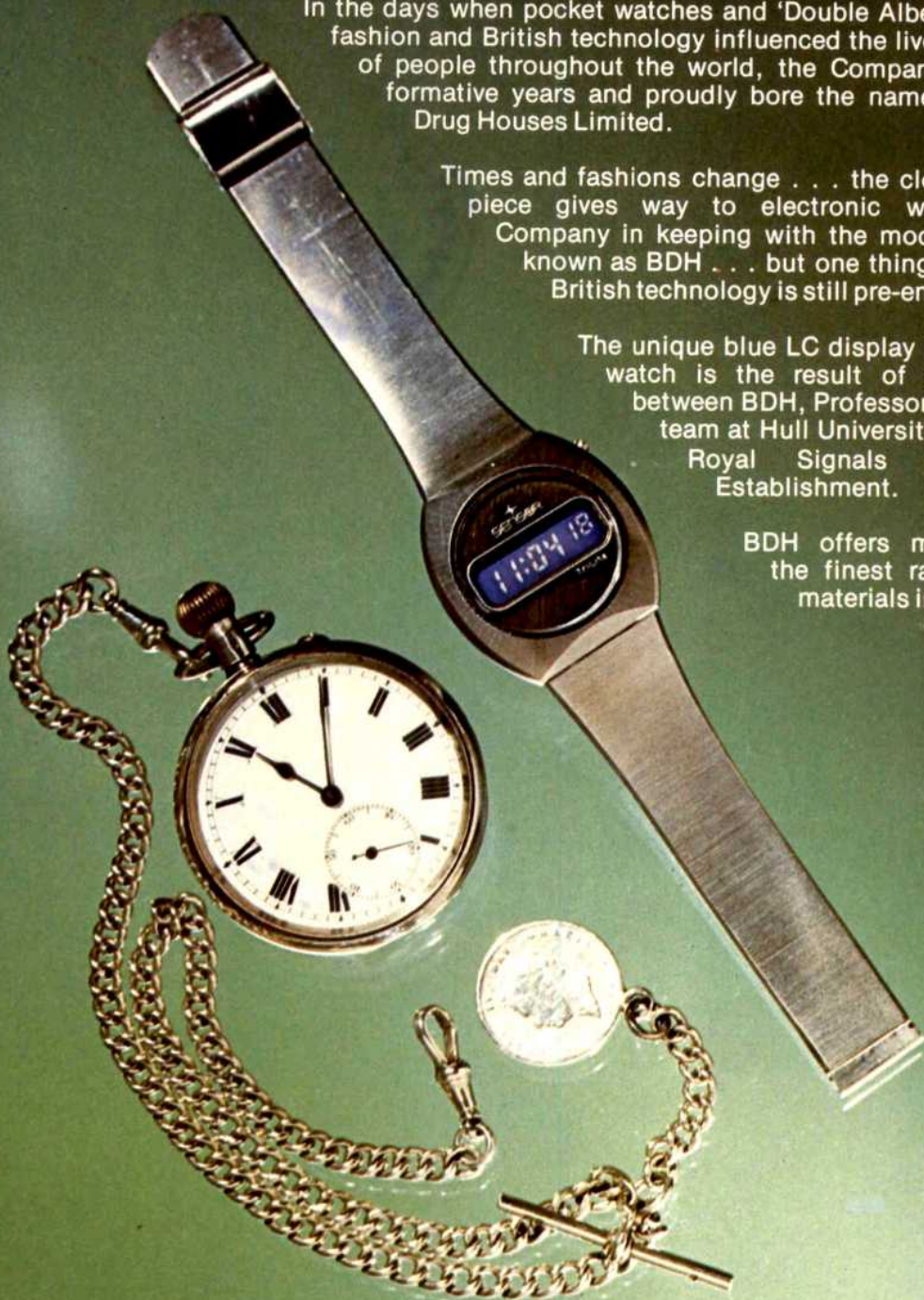
# *time flies...*

In the days when pocket watches and 'Double Alberts' were the fashion and British technology influenced the lives of millions of people throughout the world, the Company was in its formative years and proudly bore the name The British Drug Houses Limited.

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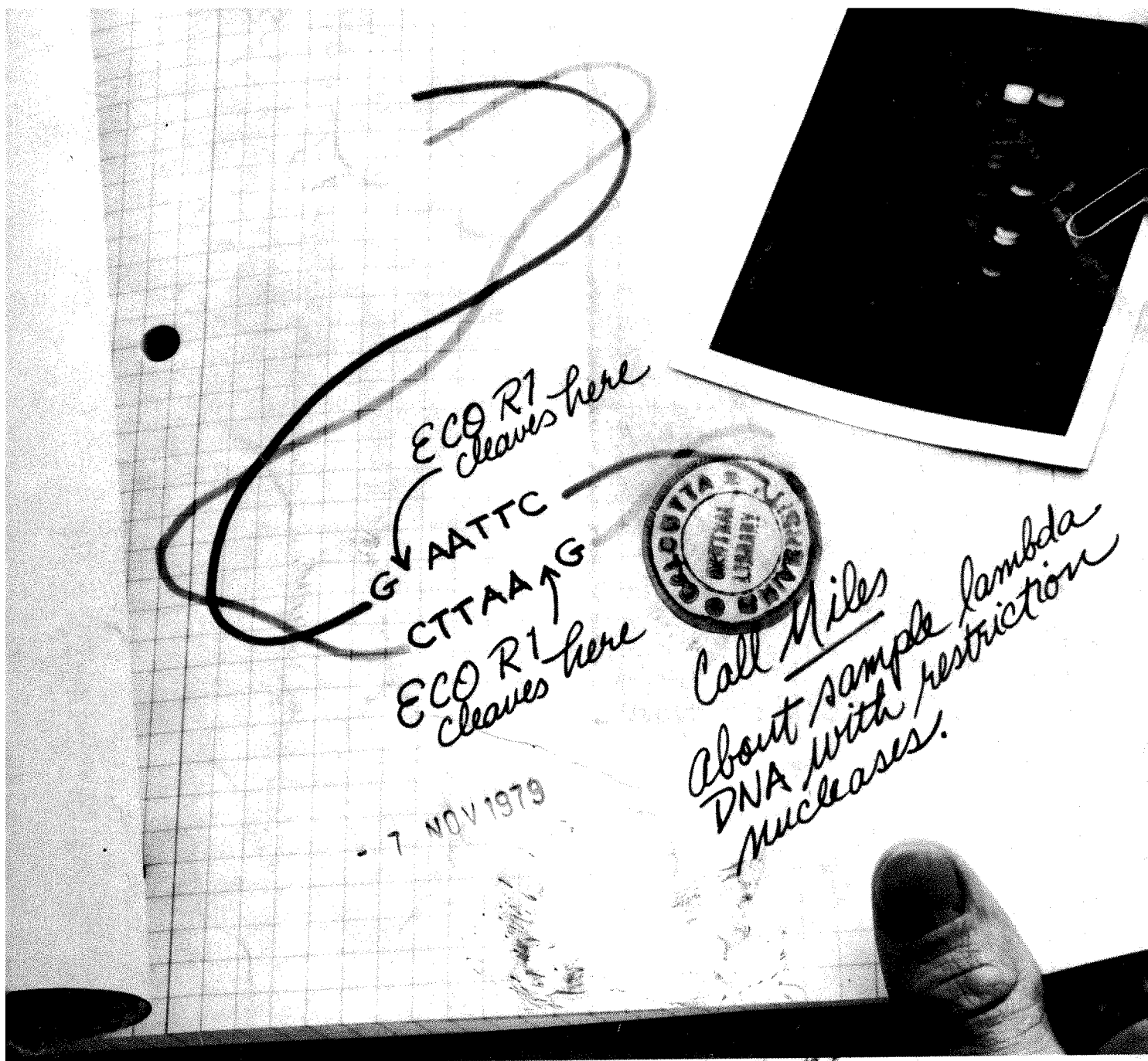


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Scanning electron micrograph of 1st instar aphids. The manner in which the gall aphid defends a micro-territory is described on page 324.

Photo: Debra L. Wright, Susan Pratt

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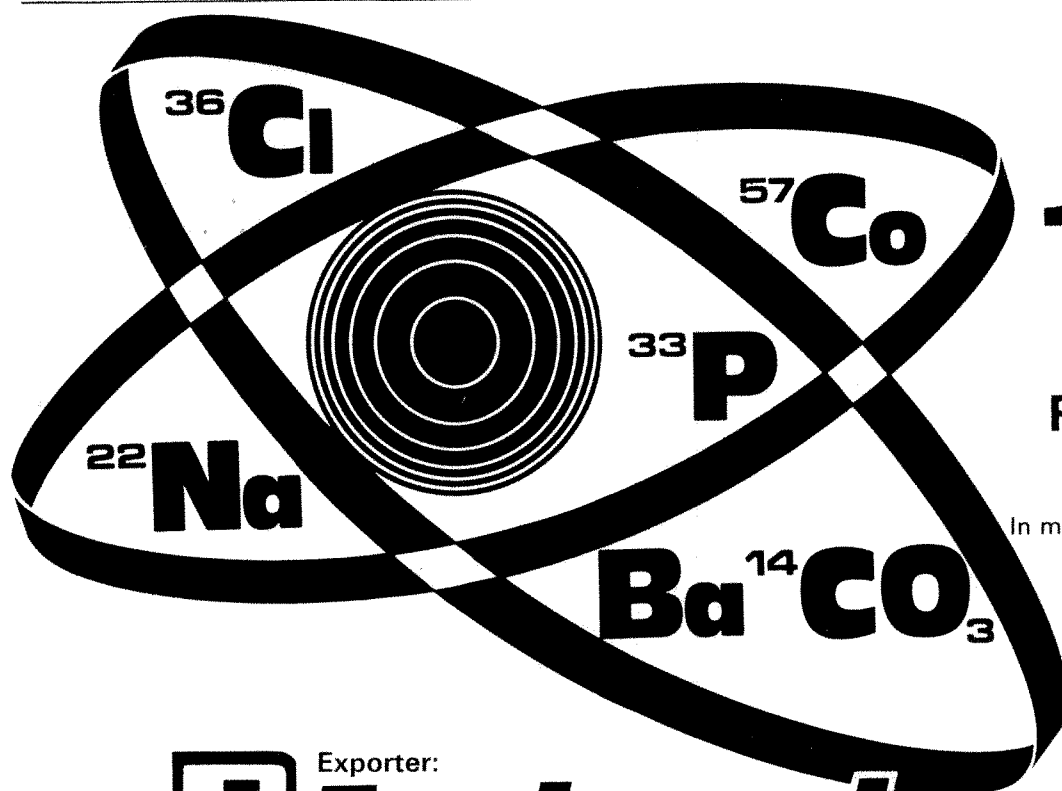
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● Articles may be up to 3,000 words long with at most six displayed items (figures and tables); they are reports of major research developments.

● Letters are brief reports of original research of unusual and wide interest, not in general longer than 1,000 words; they have at most three or four displayed items.

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**Manuscripts** may be submitted either to London or New York. Three typed copies should be submitted, each including lettered copies of figures. Typing (including references) should be double spaced. The title should be brief and informative. Pages should be numbered. References, tables and figure legends should start on separate pages. Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends. Units should conform to the *Système International*. Greek characters should be identified in the margin on their first appearance. Equations should occupy single lines if possible; exp (a) is preferred to  $e^a$  if 'a' is more than one character.

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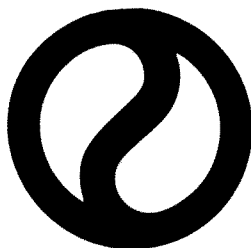
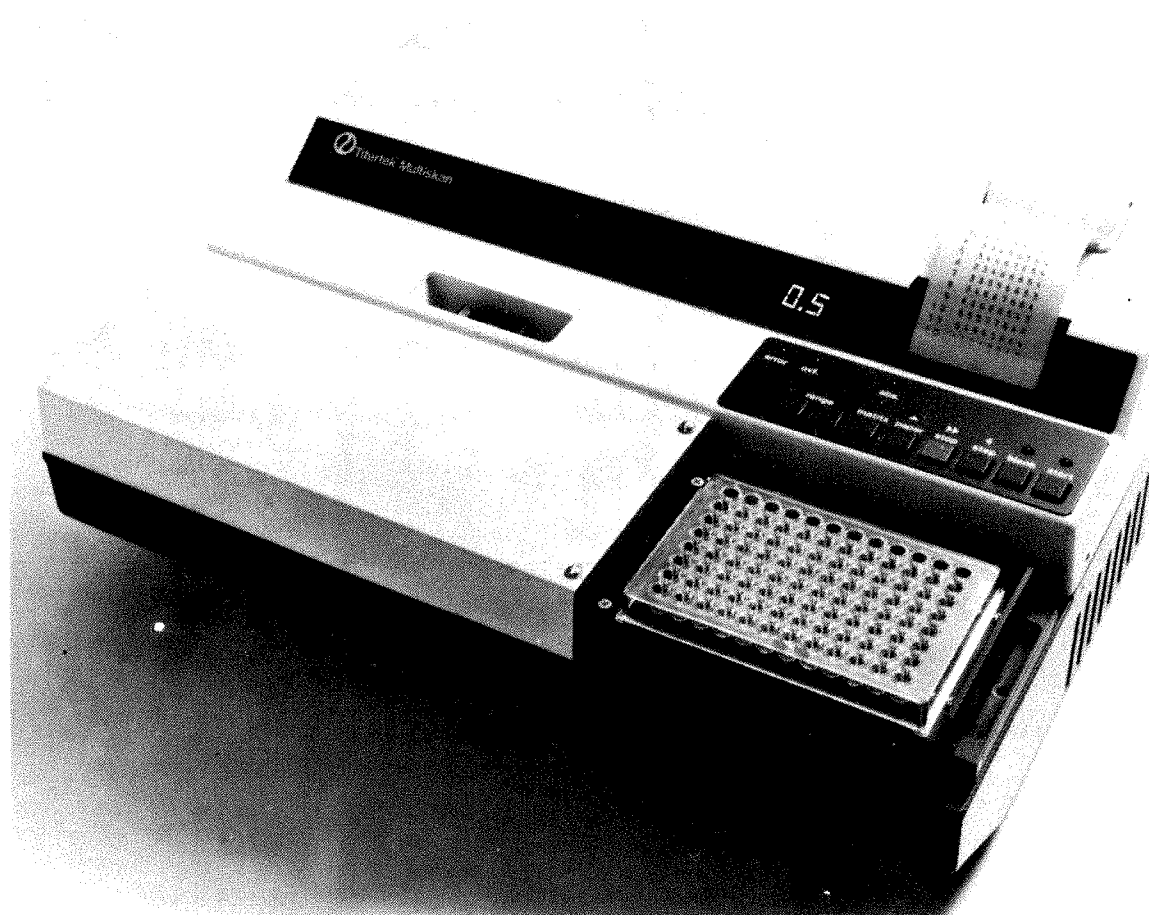
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## A select committee to be kept

WITHIN a period of weeks rather than months the UK House of Commons is likely to start discussing changes in procedure, and high on the list of priorities will be a re-organisation of the select committee structure. There is a wide measure of support in parliament for the re-aligning of committees along ministerial lines, and were this to come about the Select Committee on Science and Technology would be disbanded; scientific issues would then be the concern of, at the least, the select committees on agriculture; defence; education, science and the arts; energy; environment; industry; and social services. Professors Ziman and Denbigh recently expressed the view of the Council for Science and Society that this would be a retrograde step (10 May, p 100).

This is a view which will be widely shared by scientists and technologists. The present committee has come in for its fair share of criticism from us and others in the past, but at least it has provided a fairly effective way by which the communities of science and technology could make contact with parliament, in an almost totally non-partisan way, on matters of concern. In none of the proposed new

committees will scientists find their interests and worries given anything like the same attention—least of all in the education, science and arts committee, where education will surely swamp everything else.

The scientific and technological community, almost by its very nature, has never had more than a handful of its number in the Commons. During its time, however, the select committee has turned up several non-scientists who have taken an active and highly intelligent interest in scientific matters; in the new order there is unlikely to be any incentive for such people to come forward.

Finally, the assumption made in the new scheme is that the way the government divides its ministerial responsibilities is the way that major national issues divide. This is simply not true in the scientific and technological field. Who would look at genetic manipulation? The impact of microprocessors? Support for innovation? Scientific manpower problems? Training for the engineering profession? Climatic change? These are all matters in which parliamentarians ought to be informed. And none of them falls neatly into one department's remit. □

## Elitist patronage—and rightly so

FIVE years ago the German pharmaceutical company C. H. Boehringer Sohn of Ingelheim invited a group of biologists, medical researchers, and philosophers to spend a few days together in a well-appointed Schloss far from the pressures of colleagues, students and telephones. The group brooded on the question of creativity, and a report of the deliberations was later published (*The creative process in science and medicine* edited by Hans Krebs and Julian Shelley; Excerpta Medica).

The indefatigable Dr Shelley, Boehringer's Director of Clinical Investigation, has just stage-managed a second symposium with a broad interdisciplinary sweep. As if the first of them were not wide-ranging enough, the second was entitled 'Structure in Science and Art', and this time the biologists and philosophers were joined by an architect, a professor of English, two composers, a novelist, three physicists, a concert pianist and an art historian. This heady brew resembled nothing so much as a High Table of High Tables in the Oxbridge tradition, except, of course, that with a battery of microphones and an army of typists waiting to transcribe every slightest remark, conversation was considerably more elevated than standard High Table fare concerning the foibles of students, the price of books and the run-down condition of the college gardens.

This is no place to try to summarise the presentations and discussions, which roamed over perception, the ob-

server in the universe, structuralism in the novel, D'Arcy Thompson's mathematical analysis of form, Breughel's 'Icarus', Schoenberg's piano music and much else besides. But it is appropriate to ask whether at the end of the day any threads, however tenuous, had been laid across the yawning chasm between the sciences and the arts. Does 'structure' offer any common ground—does, for instance, appreciation of the structure of a Mozart concerto help those looking for structure in biological molecules? On the face of it, no.

Those who read the proceedings of this symposium in the hope of seeing new lines of thought emerge, spanning the disciplines, will almost certainly be disappointed. But sensitivity to a wide range of subjects probably does help to enrich work in one's own particular discipline. And occasions such as this symposium do help to heighten that sensitivity of those fortunate participants.

It is a commonplace to say of a conference that the real work was done outside the formal sessions. In this case, however, there is little doubt that this was true; the new contacts, the informal conversations will count for more than what was actually said to the microphones. Mercifully the sponsors seem to appreciate this, and look for no agreed conclusions, no statement to the world out of it all. It is enlightened patronage, of an elitist sort that few dare to pursue these days. □

## A week of nuclear decision in the United States and Europe

# Columbia University drops plans to operate reactor

LAST week the University of Columbia in New York withdrew a request for a permit from the City of New York to operate a teaching reactor built on the university's Morningside campus in 1969. The university also agreed to withdraw a federal lawsuit, filed jointly with the Department of Justice, challenging the city's jurisdiction over nuclear reactors and radioactive materials.

This move follows a decision by the Faculty of Engineering at Columbia University to accept a request from the president of the university, Dr William J. McGill, not to put the reactor into operation.

Dr McGill had previously resisted moves by members of the local community to prevent the reactor from operating because of health dangers which, some had claimed, would be posed in the case of an accident. However, according to a statement made at a public hearing two weeks ago, Dr McGill said that he had changed his stance mainly in response to public reaction to the Three Mile Island nuclear accident. The university's faculty of engineering has agreed to "acquiesce" to the president's decision. Individually, however, many faculty members disagree with the action, claiming that only increased familiarity with reactor technology will enable operators of the future to avoid the type of mistakes made at Harrisburg.

Students at the university, on the other hand, who have been campaigning against the reactor for many years, claim that the action does not go far enough. They want the reactor, which was constructed in the 1960s but has never been operated due to a succession of legal disputes, to be dismantled, and effort to be transferred to solar energy research.

The teaching reactor is a Triga Mark II reactor, and is licensed to operate up to 250 kilowatts, although university officials say that it would only run at an average of 1 kilowatt. The reactor itself cost about \$500,000, and was financed by the National Science Foundation, with another \$500,000 being spent on the surrounding building. Similar reactors are already in operation in about 60 universities in both the US and overseas.

Earlier this year, after a series of legal battles over who should have the right to licence the reactor Dr McGill announced that the reactor would still go into operation but not until all the legal aspects had been resolved.

Following the Three Mile Island accident, however, and subsequent

student demonstrations on the Columbia campus, Dr McGill announced that he was going to ask the faculty of engineering to reconsider its plans to operate the reactor. The opposition to the university reactor, which he had previously envisaged to be a political problem, "was clearly revealed during the last week in March and the first week of April as a human problem of mounting seriousness," Dr McGill told the public hearing in New York.

"The Triga issue suddenly acquired the potential to tear the campus apart in an explosion of fear and apprehen-

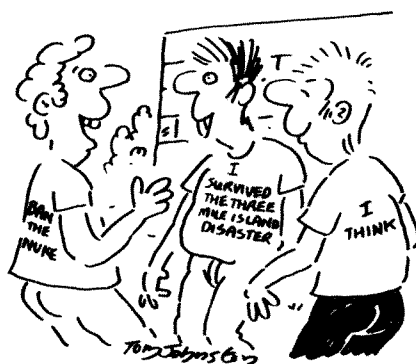
sion. The larger interests of safety and well-being affecting everyone at Columbia University required that I act immediately, and I did so."

Faculty members, while agreeing to go along with the president's decision, are critical both of its implications, and of the way that the decision was taken. "The president's decision was a political one not an educational one; the decision not to operate the reactor is a loss to the country and to the educational community that we cannot afford at the present time," Professor William Havens, director of the university's nuclear science programmes said last week.

The reactor may still be operated in the future, if the general opposition to nuclear power should change, according to Dr Peter J. Likens, dean of the university's school of engineering. "We might seek to reconsider that decision and activate it if the social circumstances mitigate it."

Meanwhile students taking nuclear science and engineering courses at the university may be sent out to the medical research reactor operated by the Brookhaven National Laboratory on Long Island to gain first-hand experience of reactor technology.

David Dickson



"If there's no reactor to learn from, there'll be nobody to build them!"

## Punitive damages for Silkwood contamination

IN a decision which could have far-reaching implications for the US nuclear industry, a jury in Oklahoma City last Friday awarded damages of \$10.5 million to the three children of Karen Silkwood, an employee of the Kerr-McGee corporation who, it accepted, had been contaminated by plutonium while working in the company's nuclear fuel processing plant.

Miss Silkwood, a laboratory technician in the plant and an activist in the Old Chemical and Atomic Workers Union, was killed in a car accident in 1974 on the way to a meeting with a newspaper reporter at which, she had said, she would produce evidence that Kerr-McGee had carelessly exposed employees to plutonium. After the accident investigators found traces of plutonium in Miss Silkwood's apartment but no papers were found in the car in which she had been travelling.

After a 10-week trial, the jury found the company negligent in the contamination of Miss Silkwood and her apartment, and rejected the charge that she had contaminated herself with plutonium stolen from the plant in

order to incriminate the company. They awarded Miss Silkwood's estate \$505,000 in actual damages, and \$10 million in punitive damages. During the trial, attorney's for the estate argued that working conditions inside the plant were inherently unsafe, and that workers had not been told that plutonium could cause cancer.

Company officials reported that they had informed the Atomic Energy Commission that they had been unable to account for about 40 lbs of weapons-grade plutonium, and believed that the material was trapped in pipes in the plant; the prosecution attorneys contended that the company's inability to account for the plutonium was further evidence of its negligence. The Kerr-McGee plant at which Miss Silkwood worked has since been closed down.

Agents for Miss Silkwood's estate are bringing a further lawsuit against the Oklahoma City Police Force, the FBI and Kerr-McGee concerning the circumstances of her death and a high OCAW official had called for a special government prosecutor to re-open the case. □



# Switzerland increases control of nuclear power

THE referendum on the law governing atomic power in Switzerland last week—at which voters approved the government's revision by more than two to one—was hardly the centre of a historic debate. In comparison to the heated debate before the 18 February vote on the nuclear issue—which by a narrow margin upheld the old law rather than one which would have prevented further development of nuclear power—the political temperature has reached an all-time low. All the major parties and organisations either recommended voting for the revision, or refused to take up positions. There has been no propaganda clash. The newspapers are practically void of announcements, and the usual stream of press conferences and meetings is absent.

The main reason for this lack of interest is that the revised law represents a compromise which satisfies no-one. The nuclear lobby is unenthusiastic about the restrictions but reckons "it could have been worse". The only positive aspect it discerns is the clarification of the waste disposal problem (5 April). The anti-nuclear group's general attitude is "better than nothing", although one section voted no, on the grounds that if the revision was rejected, a new revision could only be even more restrictive.

The proposals embodied in the revised law contain enough seeds of discontent to ensure that the peace will probably be only a lull in the storm.

- The granting of skeleton permits for new nuclear power stations, and of construction permits for those already planned—both to be done now by parliament, rather than the cabinet alone—will depend on:

- the demonstration of electricity demand; and
- safe nuclear waste management.

That the law leaves much latitude in the interpretation of these points became clear at a series of hearings in Bern organised by the Swiss Energy Foundation, the main "soft energy" group and leader of the environmentalist opposition to nuclear development. Officials of the various government de-

partments which will be required to put the revised law into practice were interrogated by representatives of both the pro and anti-nuclear groups on their interpretation of the demand and waste management paragraphs, as well as on the proposed procedure for nuclear power stations which already have site but not construction permits.

The transcripts of the hearings<sup>1</sup> indicate that the "demonstration of demand" will become a hard-fought issue in future. How can demand be demonstrated when Switzerland is literally "throwing away" one-third of all the energy it produces because of inefficient systems, poor insulation, lack of incentives for energy saving, etc.? Is not the level of demand really a political question? What reserve capacity is needed to meet short-term abnormal demands or break-downs in functioning units? To decide these questions it is proposed to set up an Energy Commission, but the problem is how to make sure that it is unbiased and uninfluenced by the powerful lobbies.

The "demonstration of safe nuclear waste management" also leaves many questions open. Will it be possible, even with the revised law, to test drill for underground repositories against the will of the local population, without excessive delays? What does safe mean, and how can safety be demonstrated in future waste management when so much of the process takes place outside the country—is what is safe for them, safe for us? Is the question of disposal, particularly of high-level waste, so controversial among scientists that it can hardly be assessed by the present Waste Management Working Group, largely made up of civil servants? How can a waste disposal project be worked out within the next five years (when the first power station falling under the new regulations will be finished) when other countries ahead of Switzerland in R & D envisage 10 more years at least?

Geoff Milnes

<sup>1</sup>"Atomgesetzrevision durchleuchtet. Ein Hearing" SES-Report No. 7, Schweiz. Energie-Stiftung, 8001 Zürich.

## Gorleben nuclear waste facility scrapped

In West Germany last week the premier of Lower Saxony, Herr Ernst Albrecht, announced that he will not grant approval for the building of a nuclear waste reprocessing and disposal facility at Gorleben. Speaking on television he said the decision was not a technical

one—he believed that the plant would be safe—but he had to take account of the weight of public opinion. However, test drilling in salt domes at Gorleben (see below) will continue.

For a detailed review of the decision, see page 283.



## EEC Commissioner opens European nuclear fusion centre

DR GUIDO Brunner, member of the European Commission for Energy, Research, Science and Education, laid the foundation stone for JET, the Joint European Torus, at the Culham Laboratory, UK, last week. During the ceremony, Dr Brunner described JET as a "leading project in the world" in the field of fusion research.

JET's world lead, however, has been

eroded by the delays to the start of the project caused by the two year negotiations in 1975-77 between EEC member states over where it should be sited. The delay has given its nearest rival, the Tokamak Fusion Test Reactor, at Princeton University, US, an eighteen month lead.

The total cost of building JET is estimated at about 200 million Euro-

pean Units of Account (at January 1979 prices). The date for completion is set at the end of 1982 and subsequent annual operating costs are expected to be about £20 million at today's prices. The JET team, which will be drawn from fusion laboratories all over Europe, is expected to be about 320 strong including 120 scientists and engineers. At present

130 people are employed on the project, half of them under Euratom contract, and half of them by the UKAEA which runs Culham.

According to Dr Hans-Otto Wüster, Director of the JET Joint Undertaking and Dr Paul Rebut, leader of the JET design team, there are some worries that JET will not attract enough high calibre fusion physicists to make up the rest of the team. One problem is that during the lengthy debate over where to site the project, almost half the design team returned to their home laboratories. Although many of these have now returned to JET, it is feared that the salaries being offered by Euratom are insufficient to entice French and German scientists to the Oxfordshire countryside.

Initially, experiments on JET will be with a D-D plasma. If these are successful, then the project will move into the "radioactive stage" by 1983-84. This will involve beam-plasma D-T operation resulting in neutron production which will activate the walls of the vessel containing the plasma. Dr Wüster said that this stage of the experimental programme would not commence without further agreement. He also assured that the radioactivity produced would be between ten and a hundred times less than that produced by nuclear fission. As the amount of radioactive material created would be very small, he thought that energy produced from fusion could prove "environmentally advantageous".

The decision over whether or not to enter the 'radioactive stage' is bound to be difficult, according to Dr R. J. Bickerton. There is already one school of thought which would like to proceed to reactor conditions as quickly as possible and another that would rather understand the plasma physics thoroughly first. This difference in opinion is to some extent reflected in the discussions which have already started on the machine to follow JET. Dr Bas Pease, Director of Culham Laboratory, thinks that the next machine should aim at demonstrating a net production of electricity. "I favour the view that we should attempt this after JET, but I can't yet say that I have persuaded a majority of colleagues of that view", he said.

The Euratom countries have already set up a Next European Torus Study Group to discuss the next machine. According to Dr Brunner, however, Europe may not be able to afford to build JET's successor alone. It is therefore cooperating in the INTOR Study Group, under the auspices of the International Atomic Energy Agency, which is looking at the possibility of the USSR, Western Europe, the US and Japan building a worldwide machine.

Judy Redfearn

## US foreign research rises to \$1.5 billion

THE vast majority of research carried out by US private corporations in foreign countries is concerned with developing products for local market conditions, rather than with longer term basic or applied research goals, according to a survey conducted by the National Science Foundation.

Research and development carried out abroad by US corporations increased by 41% between 1974 and 1977, to a total of \$1.5 billion, the survey reports. This represents about 7% of the total expenditure on R&D by private companies, which increased by 32% over this period.

The most substantial increase in overseas R&D occurred in the pharmaceutical industry, with many companies conducting trials of new drugs abroad in order to take advantage of more liberal conditions on foreign testing introduced by the Food and Drug Administration in 1975. Thus between 1974 and 1977, the amount of R&D conducted by pharmaceutical companies abroad more than doubled, compared to an increase of only 34% in the funds spent at home over this period (although the report adds that foreign and domestic R&D are now increasing at about the same rate).

The NSF survey says that in companies with world-wide research capabilities, the US laboratory usually takes the lead in overall technological development, with the foreign labora-

tories providing specialised development for particular market conditions. The foreign R&D facilities of these companies therefore conducted primarily development projects, even though basic and applied research accounts for almost one quarter of total domestic R&D spending by US companies.

Interviews carried out with the managers of 18 large private corporations revealed only one case in which a company reported any expenditure on basic research conducted outside the US. Two other companies reported that their foreign research facilities carried out some applied research work.

Looking to the future, the NSF study says that an expected increase in overseas sales by US corporations is likely to cause such companies to open new foreign R&D facilities or to expand existing operations, with increased sales, rather than other factors such as lower research and development costs, providing the main motivation for such developments.

The report also notes that several R&D directors outside the pharmaceutical industry said that increasing regulation by bodies such as the Occupational Safety and Health Administration and the Environmental Protection Agency in the US would tend to cause companies to move R&D resources to other countries where operations would not be affected as much. □

## University of Houston expels professor

THE University of Houston in Texas is refusing to renew the contract of Professor Archer J. P. Martin, joint winner of the Nobel prize for chemistry in 1952 with R. L. M. Synge for his work on chromatography, on the grounds of "inadequate productivity".

Professor Martin, who is 69, was appointed to the Welch chair of chemistry in 1974, and until last year held a joint appointment at the University of Sussex, where he was carrying out research on protein separation sponsored by the Medical Research Council.

The University of Houston requires that after a faculty member reaches the age of 64, his or her tenure must be renewed annually. Last year the university told Dr Martin that the Department of Chemistry did not consider he had published enough scientific papers, and that his appointment would not be renewed.

Professor Martin told *Nature* last week that when he took the post, he had not been aware that there would be little money for research assistants or experimental facilities and consequently that he spent most of his time in Houston planning experiments to be carried out in Sussex.

He accepts that he has published very few scientific papers over the past five years from his work at Houston, but claims that he has no desire to rush into print unnecessarily (his total scientific output is about 70 scientific papers). According to Professor Martin, the university did not take into account papers produced from the work at Sussex in considering whether his contract should be renewed.

A faculty committee, to which Professor Martin protested at his dismissal, came to the conclusion that the terms of his appointment had not been initially made adequately clear, and recommended that he be kept on the staff. However, two weeks ago Professor Robert H. Walker, Dean of the College of Natural Sciences and Mathematics, informed Professor Martin that the university had no power to keep him on unless the Department of Chemistry changed its mind. According to Dr. Walker, the university does not set any specific target on the number of papers which a research worker is expected to produce each year but, he says that there is nevertheless "a certain expectation of productivity". □

# Congress goes easy on science—so far

A KEY subcommittee of the US House of Representatives last week recommended that \$19 million be cut from the budget request for the National Science Foundation which had been submitted by President Carter. Although this represents a reduction of almost 2% in the \$1,006 million request submitted. It is considerably less than the \$44 million reduction which the same subcommittee wanted made in the NSF's budget last year.

The subcommittee's recommendations, which have to be accepted by the full committee of Appropriations before being debated on the floor of the House, would delete \$10 million from the agency's basic research programmes out of a requested total of \$793.3 million, \$3 million out of the \$62.4 million requested for the applied science and research applications programmes, and \$3 million out of programme management.

The subcommittee made no mention of the ceiling which Congress placed on salaries of NSF grant-holders last year, leading some to speculate that the ceiling might be lifted during conference with the Senate. However, neither did it add any extra money for research on earthquake hazards, for which the House last month authorised spending to be increased from \$18.3 million to \$22.8 million.

NSF officials were relieved that the cuts were not greater (a move to make an additional \$12 million cut is said to have been headed off in the subcommittee meeting). However, the real tests will come in the middle of next month when the NSF request is debated both on the floor of the House—where the Appropriations Bill has already been cut back by \$14 million—and in the Senate Appropriations Subcommittee chaired by Senator William Proxmire. □

Reports  
by  
David  
Dickson



## Funds denied for nuclear waste

THE House Armed Services Committee has voted to cut off all funds for an experimental project to store nuclear waste in salt caverns near Carlsbad, New Mexico.

The project, known as a waste isolation pilot plant (WIPP) has been under development by the Department of Energy for some time. The site was chosen in 1976, and if permission to build the plant is granted, the site could be ready to receive both transuranic wastes from the defence programme and commercial waste from nuclear reactors within a few years.

The scheme has generated intense controversy in New Mexico. Supporters say the WIPP facility would bring new jobs and extra income to the area. Opponents claim it would restrict access to other valuable resources, such as potash, and also point to doubts over the suitability of salt as a disposal medium.

The House Armed Services Committee voted against further funding for the WIPP facility not on environmental or scientific grounds, but because it felt that a plant initially intended for defence wastes should not also be used for the disposal of commercial wastes. Meanwhile, the Senate Armed Services Committee has agreed to let funding for the project stand in the Department of Energy's budget request, but on the understanding that no commercial spent fuel should be stored at the WIPP site.

The outcome will depend on what happens in floor debates and during the conference between the House and the Senate. Another factor will be the President's announcement soon on federal waste disposal, following a report on nuclear waste management prepared last year by an interagency review group.

Meanwhile, the Senate and the New York State Assembly announced last week a curb on the amount of nuclear waste that can be stored in the state's West Valley facility, which has been closed since 1972. □

## Senate committee kills fast breeder

A NEW political confrontation over the future of the liquid metal fast breeder reactor project at Clinch River in Tennessee seems certain after the Senate Energy and Natural Resources Committee last week accepted a proposal from Senator Dale Bumpers of Arkansas to kill the \$2.6 billion project. The House Science and Technology Committee has already voted to continue.

President Carter has consistently been opposed to the plan, initially on the grounds that the use of plutonium in fast breeders increases the chances of nuclear proliferation. More recently, the President has claimed that the Clinch River design was obsolete after new advances in technology, and that with a slowing down in growth of energy demand, the need for fast breeders is not so urgent.

The General Accounting Office, however, which carries out studies of particular issues for the Congress, disagrees with the President. In a report published earlier this month, the GAO stated that the Clinch River project would be a "logical and prudent step".

In pledging its support for the project, the House Committee added \$184 million to the authorisation request for the Department of Energy so that the project could proceed. Senator Bumpers' proposal, which has been accepted by the Senate Energy Committee, is to terminate the breeder project by 1 October, but to authorise a design study for a larger breeder test plant. "Clinch River is dead and the sooner we admit it, the more the American people will applaud us for doing it," Senator Bumpers said. □

## Technical aid restored to UN bodies

THE US Senate voted last week to repeal a decision, taken in the heat of the closing hours of the 95th Congress last autumn, which effectively cut off all US contributions to bodies in the United Nations system.

The earlier decision took the form of an amendment, proposed by Senator Jesse Helms of North Carolina, which specified that none of the funds which the US contributes to bodies such as the World Health Organisation and the Food and Agricultural Organisation could be used for the purpose of "technical assistance".

The UN bodies subsequently told the US State Department that it could not

accept contributions under those conditions. Last week, however, in passing the Foreign Relations Authorisations Act for 1980, the Senate in effect repealed the Helms Amendment, following a similar move in the House of Representatives.

The Senate also defeated, by 54 votes to 35, a further amendment from Senator Helms which would have revived the issue by refusing to include money for technical assistance in the mandatory contributions which the US pays to the UN bodies. Senator Helms said it was a matter of whether the Senate wished to impose an 'International Tax' on Americans. □



# Pesticide resistance on the increase, says UNEP

RESISTANCE to pesticides has been spreading so rapidly among pests that no manufacturer cared to offer a new pesticide to the World Health Organisation for safety testing in 1978. It now takes millions of dollars to test the safety of new chemicals and often within months pests start developing resistance to it. WHO recently reported that it is now cutting down its field staff involved in safety testing of pesticides.

Reflecting these events, this year's State of the Environment Report to be released by the United Nations Environment Programme on the occasion of the World Environment Day (5 June) will focus upon the increasing dangers posed by pesticide resistance. The report makes quite frightening reading.

It points out that in 1965, the Food and Agricultural Organisation listed 182 resistant strains of arthropod pests; in 1968, it listed 228 resistant species; and now its latest survey of 1977 lists 364 species.

There are now 223 agricultural pests resistant to nine of the major groups of pesticides. Many of these, says the UNEP report, "are major pests of major crops, such as cotton bollworm, the boll weevil, and the leafworm of cotton, the rice stem borer and the brown plant hopper, the Colorado beetle of potatoes, spider mites of fruit and glasshouse crops, and cutworms and weevils of cereals".

Up to 1970, very few resistant plant pathogens had been observed. But with the introduction of new systemic fungicides, the resistance problem has crept in, and now more than 35 species of plant pathogens have been reported as resistant.

Rodents, which destroy crops, are also becoming resistant to rodenticides. Latest FAO figures show that seven species of rodents, including two important and widespread species, *Rattus rattus*, and *Rattus norvegicus*, have developed resistant strains.

If there is any ray of hope it seems to be with weeds, which have not yet shown any sign of developing genetical resistance similar to that occurring amongst insects—even though herbicides now account for some 50% of all pesticides applied. For long-lived perennial and vegetatively reproducing weeds, says the report, "the potential for development of genetical resistance seems low bearing in mind that experience with insects and other classes has demonstrated that in the field many generations must pass before such resistance reaches notice-

able levels".

The most worrying situation is in the field of public health. WHO figures cited in the UNEP report show that there are now 121 resistant strains of insects important to public health campaigns compared to 102 in 1968. In 1969, 15 species of anopheline mosquitoes were resistant to DDT and some 37 to dieldrin. In 1976, some 43 species were known to be resistant to dieldrin, 24 to DDT, five to organophosphates like malathion, and two to carbamates.

Resistance to insecticides is now found amongst anopheline mosquitoes in 62 countries out of 107 where there is malaria. In several parts of the world there has been a massive resurgence of the disease, with some countries showing a 30-40 fold increase in the number of malaria cases since 1968-70.

Switching over from DDT to other insecticides like malathion has not proved easy. Most of the substitutes of DDT are both much more toxic and much more costly. India's malaria control programme alone consumes nearly 60% of the Indian government's health budget.

Amongst culicine mosquitoes, vectors of such diseases as yellow fever, filariasis and dengue, resistance has increased from 19 species in 1968 to 41 species in 1975. Other important vectors such as houseflies, black flies and fleas are also becoming resistant. The house fly, says the UNEP report, seems to be the insect "with the greatest ability to develop resistance to insecticides over the widest geographical area". A total of 121 resistant strains of housefly were reported in 1975.

Research on pest control agents with novel modes of action like chemosterilants, hormones and growth inhibitors, and biological agents like bacteria, viruses and fungi, has been hailed in the belief that pests would be less likely to develop resistance to them. The UNEP report however points out that this hope is ill-founded.

Resistance has already appeared to hormone-based pesticides where little resistance had been expected. "New compounds such as growth regulators and microbial pesticides have not been in use long enough or on a wide enough scale to show perceptible resistance, but here again as with chemosterilants, it has been possible in the laboratory to develop resistance by artificial selection."

The flour beetle, *Tribolium*, has not



only developed multiple resistance to conventional pesticides but also acquired significant cross-resistance to the growth inhibitor, methoprene. It has also been shown that houseflies can become resistant both to the spores of *Bacillus thuringiensis* and to its toxin. *B. thuringiensis* is expected to become an important biological control agent.

What then is the solution to pest control? UNEP points out that the classical alternative approach of changing the pesticide when faced with the problem of resistance is a practical solution only in the short term. Alternative pesticides need to be developed but in the long term, the situation is such that it requires the development of alternative strategies.

Instead of total reliance on just one type of control agent like chemical pesticides, UNEP favours the concept of 'integrated pest control.' This would include elements of chemical pest control, environmental control of breeding habitats, genetic control techniques, biological control, behavioural control using sex pheromones and related compounds, and breeding of resistance in crops and animals.

Integrated pest control strategies will have to be specific to a pest in a specific environment. This will require considerable research. An FAO/UNEP panel of experts on integrated pest control has over the last few years developed a Global Programme for Integrated Pest Control in relation to various priority crops like cotton, rice, sorghum, maize, millet, roots and tubers and grain legumes, and is now extending this to vegetable crops.

Studies conducted in South America using integrated pest control techniques on cotton—the highest consumer of insecticides in agriculture—shows that the quantity of chemicals needed can be reduced by a third, thus cutting down costs and health risks and increasing productivity. But while this system of integrated pest management has been considerably developed, both in theory and in practice, as regards agricultural pest control, the UNEP report claims that extensive research programmes are still needed to apply it to public health vector control.

The switch from chemicals to the new packages of integrated practices is therefore likely to take a considerable time.

Anil Agarwal

# Ecology groups combat Chinese irrigation plan

CHINESE plans to divert water from the Yangtze to irrigate northern China have run into cautiously expressed but clear opposition from Chinese environmentalists. A recent meeting of the Chinese Society for Water Conservancy, officially described as an "academic discussion" on the plan, concluded that in the past, "our improper water control plans violated objective laws". Any plans to divert the Yangtze, they stressed, must be considered not from a purely engineering point of view, but should also take into account possible ecological changes produced by the scheme.

As far as the engineers are concerned, plans still remain flexible. A national forum held in Tianjin at the beginning of April discussed three possible routes: the "Western route" from the mountainous upper reaches of the Yangtze via "snaking tunnels" to north-west China; the "middle route", via a projected canal which would cross the Hwang-Ho near Zhengzhou; and the "eastern route" which would roughly follow that of the Grand Canal built by the Emperor Yang Di in about 600 AD.

All three routes, the forum decided, were practicable, although each plan still required some improvement. Construction of the western route, it was concluded, lay beyond China's capability at the moment. The eastern route, first announced in August 1978, although feasible, attracted considerably less support than the middle route, which appeared to be the choice of the Yangtze Basin Planning Commission. A spokesman for the commission told the forum that this route would involve the construction of a 1,000 km canal from the Banjiangkou reservoir on the Han river (a major tributary of the Yangtze), as far as Peking, via the foothills of the Funiu range and the Nanyang basin in southern Henan, approaching Peking on a route parallel to the Peking-Canton railway. When completed, he said, the scheme would irrigate 4,666,000 ha of farmland, bringing an average of 10,000 million m<sup>3</sup> water annually to the Hai river basin, thus equalling the annual flow of the entire Hai system.

However, it might seem strange to divert water from the Yangtze across the Hwang-Ho. Why not simply irrigate north-west China with water diverted from the Hwang-Ho? The reason is that the mean flow rate of the Yangtze



is 34,000 m<sup>3</sup> sec<sup>-1</sup>, considerably greater than the 1,500 m<sup>3</sup> sec<sup>-1</sup> flow rate achieved on average by the Hwang-Ho.

The spokesman admitted, however, that there was one major disadvantage to this plan. The annual output of the Danjiangkou hydroelectric station would have to be reduced by anything from 700 million to 1,700 million kWh annually. This, however, he claimed, could be compensated by the Gezhou power station now under construction further westward.

At least one water conservancy specialist, a delegate from Qinghua University, showed some support for the plan. He noted that large areas of rock fissures and sandy structures beneath Peking city could be used as an emergency water store, while some 15,000 sq km of "abandoned river beds" north of the Hwang-Ho could be used to store 39,000 m<sup>3</sup>. In general, however, the conservationists are somewhat cautious. The "academic discussion" of the Conservancy Society pointed out a number of factors which demand further consideration. Would the diversion, they asked, cause secondary salinisation and alkalisation of the soil? Further, what would happen in drought years? In 1978, droughts in the middle and lower Yangtze basin and the consumption of large quantities of water for irrigation led to a back-flow of sea water into the Yangtze estuary, so that, for a time, the population of Shanghai was obliged to drink brackish water, while at Chongming island, which supplies agricultural and market-garden produce to Shanghai, cultivation was seriously affected. Any proposed diversion of the Yangtze, they stress, "must conform to the law of nature and the economic law".

Vera Rich

## Hungarian scientists want better links with government

HUNGARY'S Academy of Sciences has adopted a new constitution which calls for greater accountability from government departments and industry.

Although in July 1969 the Central Committee of the Hungarian Communist Party affirmed, in a startling change of direction, the need for complete freedom in scientific research and an atmosphere of free and uninhibited discussion among scientists, economic demands, both national and within the Comecon framework, have led to a policy of closer integration between research and production. This the Hungarian scientists seemed perfectly prepared to accept. Last year's General Assembly of the Academy stressed the need for greater selectivity in research subjects, taking into account the current "social needs".

Nevertheless, this linkage does not seem to be operating as smoothly as hoped. There are considerable delays (running into years and sometimes decades) in the introduction of Hungarian patents and inventions in practice. This is not only due to bureaucratic delays; last summer, the president of the National Patent Office, Emil Tasnadi, told the prestigious political and literary journal *Elet es Prodalom* that the fault lay largely with the scientists. There are some 35,000 scientists in Hungary, he said, but their sole aim is to achieve academic degrees: the titles of Candidate (PhD) and Doctor bring with them, respectively, a life-long stipend of 500 and 1,000 forints per month. Since these degrees are earned through the publishing of articles and books, he said, and not by inventions, only six researchers in every thousand actually have a patent to their credit.

While not actually answering Tasnadi directly, Dr Janos Szentagothai, the president of the Academy, took the opportunity of this year's General Assembly to state the scientists' case. Links between research and production have come closer in recent years, he noted approvingly; nevertheless, the situation is by no means satisfactory. The various departments of the Academy duly make the proper recommendations to the production sector—but receive little or no feedback. Frequently, he said, the scientists do not know if the recommendations have even been discussed.

The new constitution of the Academy, adopted by the General Assembly on 11 May, and made retroactive to last April, demands that this situation should be rectified. □



**Harvard makes mathematics mandatory:** In a far reaching revision of its undergraduate curriculum Harvard University will demand mathematics proficiency from its entire student body. All Harvard entrants will be required to show proficiency within their first year in three areas: applications of the function concept to the real world; probability and statistics; and in the use of time-shared computers. The mathematics requirement will have further effects in raising the level of instruction of the new science requirements for non-science students. All students will be required to take a one semester course in a physical science ("predictive and deductive analysis and quantitative treatment of data") and a one semester course in a biological science ("descriptive treatment of complex historical or evolutionary systems"). Harvard's move, because of its great prestige in the US, is expected to have widespread implications for the whole of US higher education.

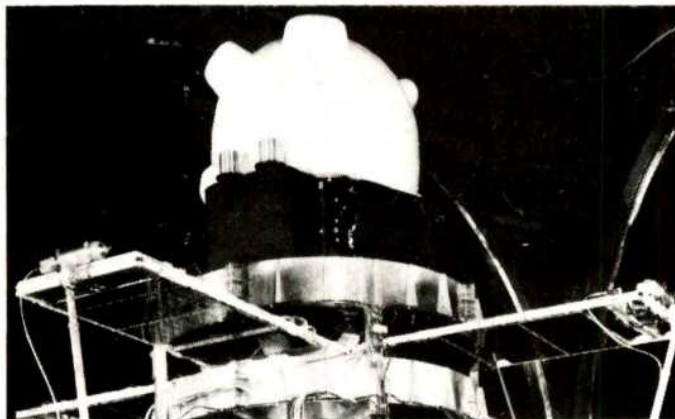
**Spanish dockers block uranium import:** The Spanish dockers union has boycotted the vessel *Covadonga* carrying nuclear fuel from the US for a power station under construction at Leimoniz in the Basque country. The boycott applies to every port in Spain and the vessel was diverted to Bordeaux. The Spanish unions appealed to unions throughout Europe for solidarity and French dockyard workers refused to handle the cargo. The ship is now reportedly bound for Cadiz in Spain. The Leimoniz power station is strongly opposed within Spain; it has been the subject of armed attacks by Basque separatists who have the support of the Left opposition and the trade union movement.

**New study challenges efficacy of nuclear energy:** A new study claims that nuclear power is "technically incapable of providing a timely and significant substitute for oil." A 50% replacement of oil by nuclear power by the year 2000 would require ordering one large power station every 3.5 days. The study, by Amory Lovins of Friends of the Earth, argues in addition that the energy supply problem is "90%" one of heat and portable liquid fuels. Lovins says that if every oil powered station in the OECD in 1975 had been replaced overnight by nuclear stations the fraction of OECD oil which is imported would have only been reduced from 65% to 60%. And at the same time there would be a greatly increased dependency on imported capital and uranium. The maximum role for nuclear power is in providing electricity which is only 10% of all primary energy. Lovins also reviews 19 studies from eight countries and estimates that conservation measures can hold steady or reduce energy needs while maintaining economic growth. Two British studies indicate that growth can triple while total energy demands could be reduced by one half.

"Is Nuclear Power Necessary?" by Amory Lovins. Friends of the Earth, 9 Poland Street, London W1V 3DG.

**Pillinger to appeal case:** David Pillinger, a biochemist made redundant at the Christie Hospital in Manchester (25 January) will take his case to an appeals tribunal. An industrial tribunal ruled against Pillinger last month finding that he had not been unfairly dismissed from his permanent post as Senior Grade Scientist at the Christie because of a lack of funding from the Medical Research Council. The appeal must be based on a point of law with no further consideration of the facts of the case. The question to be raised will "involve the whole three cornered relationship between employer, employee and granting agencies" says Pillinger. In Pillinger's case, a third party has been per-

mitted to exercise the right to hire and fire without giving reasons for its action. According to Pillinger's solicitor the case is "fundamental to the whole concept of unfair dismissal".



**UK6 due for launch this week:** The UK's sixth scientific satellite (above) is due for launch this week aboard a NASA Scout rocket. It is the last of the UK/Ariel series and carries three high energy astrophysics experiments: a cosmic ray detector for studying ultra-heavy cosmic rays; an x-ray telescope system for studying x-ray sources in the range 0.1–2.0 keV; and experiments for observing variable x-ray sources with high time resolution in the range 1.2–50 keV. UK6 will be the last purely British scientific satellite for some time at least although the UK is still negotiating with NASA over the development of a multi-mission refurbishable satellite.

**EEC takes steps to meet energy crisis:** After a delay caused by UK objections to EEC coal buying policy, the Council of Energy Ministers has approved European Community Commission research proposals covering energy saving, geothermal energy, solar energy and coal gasification. The Council has allotted 55 MEUA (£36m) over four years for energy saving projects and 95 MEUA (£63.3m) for research into alternate energy sources. In the UK, the EEC has provided 21 MEUA (£14m) for nine demonstration projects in energy saving. The applications selected under the scheme include three heat recovery projects (in steel making, plaster board manufacture, and in a swimming pool recreation complex) five heat saving manufacturing devices and a monitoring system of energy consumption.

In the meantime, the Union of European Community Industries (UNECI) has issued a report calling for a massive expenditure of 373 billion UA for investment in energy production and an additional 350 BUA for energy prospecting and for research and development. The UNECI report estimates that 650 new coal mines, six natural gas fields, and oil production equivalent to seven Niagras and 600 nuclear power stations will be needed to meet a projected doubling of energy requirements by 2000 AD.

**Skylab falls soon:** The US National Aeronautics and Space Administration announced last week that it was expecting the 85-ton Skylab space station to fall to Earth between 26 June and 9 July, with 2 July as the most probable date. According to the agency, most of the space station, which was launched in 1973 for carrying out research into activities in space, will burn up as it enters the atmosphere. However between 400 and 500 pieces are now expected to reach the surface of the Earth, scattered along a path 4,000 miles long and 100 miles wide.



# Gorleben: winning the battle, losing the war?

**Helmut Hirsch**, coordinator of scientific opposition to West Germany's nuclear reprocessing plans, gives his view of the decision last week not to proceed—for the time being

THOSE of us closely involved in the debate over whether or not to build a nuclear waste disposal centre at Gorleben have, it now seems, been barking up the wrong tree. The Federal Government stated two years ago that one of the conditions for further nuclear expansion in West Germany was what it called the "technical safety and feasibility" of nuclear processing and reprocessing. It was on this basis that the Reactor Safety Commission and the Commission for Radiological Protection expressed their opinion that the Gorleben centre was feasible. It was also on this basis that 20 international scientists, working in the Gorleben International Review, produced a 2,200 page report arguing that it was not. The matter was discussed at the controversial hearings in Hanover which ended on 3 April.

Now, it seems, we were all wrong. Because on 16 May Minister-President Albrecht of Lower Saxony revealed to the astonished German public that he was not worried about Gorleben's "technical safety and feasibility". His chief concern, he said, was its political feasibility. He turned the project down because of political, not technical, opposition.

Albrecht was well aware of the massive opposition to the centre; during the hearings, more than 100,000 people demonstrated in Hanover. He made quite clear in his speech on 16 May that he considered the roots of this opposition were wholly irrational but he decided he would not ram nuclear power down the throats of the ill-informed populace. Or at least he would not do so as long as he was the only one responsible.

Albrecht (who belongs to the CDU) is clearly very irritated about the position adopted by Lower Saxony's Social Democrats. Their leader, Ravens, came out with the conclusion that the Gorleben centre was technically not feasible. Ravens even met Chancellor Schmidt, an outspoken supporter of the Gorleben plan, but Schmidt did not pull Ravens into line. Thus, Albrecht, when announcing his decision, made it clear that he would not take it upon himself to push ahead with reprocessing plans which originated in the Federal Government's (and hence the SPD's) energy strategy but which were opposed by the SPD

in Lower Saxony, who could then profit from their opposition by gaining anti-nuclear voters.

Albrecht also announced the next step: *intermediate* storage of spent fuel elements, probably for up to 40 years, while research into final disposal is to be intensified, covering both post-reprocessing wastes and unprocessed spent fuel elements. However, it is the Gorleben salt dome—and only the Gorleben salt dome—that is to be explored for its suitability for final disposal. If the results are positive, disposal of radioactive wastes will take place there; if not, other sites will then be considered. Clearly the people of Gorleben will have to postpone their victory celebrations.

Local people fear—perhaps with good reason—that this means Gorleben is still the first and only choice for solving West Germany's nuclear waste problems, particularly if pressure mounts for an official decision on a suitable site so that the country's nuclear capacity can expand as fast as possible. They are less than confident that the exploratory drillings at



Minister-President Ernst Albrecht; turned down Gorleben plan for political reasons

Gorleben will be analysed in an unbiased manner, and fear that the salt dome will be proved suitable simply because the nuclear lobby needs it badly.

This concern is shared by Dr Friedrich Mauthe, geologist at Hanover University and one of the participants of the hearings. He says, "So far, nobody has even bothered to define clear criteria stating which drilling results are necessary for a positive judgement. I wouldn't feel too happy if the criteria are tailored after the drillings have been completed".

The procedure envisaged by Albrecht goes right against the conclusions of the Gorleben International Review, whose report emphasised that a broad investigation at several sites would be necessary before any site was singled out. The review expressed doubts about the Gorleben salt dome as well as the suitability of salt as a medium in general. The GIR proposed

detailed and timely investigations of other geologic formations. But at the hearings, Albrecht flatly refused to discuss the Gorleben salt dome.

Albrecht has avoided the question of where to site the intermediate storage facilities. This is another worry for the people of the Landkreis Lüchow-Dannenberg, to which Gorleben belongs. They might yet have to put up with storage ponds. The siting of these at Gorleben would further suggest that the nuclear industry still wants to proceed with the original proposal, with some modifications, and use step-by-step tactics to avoid major conflicts. Considering the determination with which the Federal Government has pushed reprocessing up to now, these suspicions may not be completely unfounded.

Given the uncertainty and political manoeuvring, who can blame the people of Lüchow-Dannenberg if they take things into their own hands? On 10 May, 600 local inhabitants walked into a closed meeting of local politicians discussing the Gorleben issue at Hitzacker, frustrated with the attitude of their representatives, many of whom still have not made up their minds. One farmer shouted: "We do not want the plant, and we do not like it better if it comes step by step!"

Activists collected more than 20,000 signatures in just over two weeks—in a district with 48,000 inhabitants, including children—and are still working to complete their list. The official farmers' union has come out with a clear "no" against nuclear facilities. The resistance is strengthened by the fact that test drilling has been going on at the site more or less continuously for the past two months. The latest news was that drillings had revealed a layer of peat 50–70 m below the surface. This is not unusual over a salt dome, but it indicates that the ground is not suitable for large concrete buildings.

The people of Lüchow-Dannenberg were not surprised by Albrecht's decision. Marianne Fritzen, one of the leaders of the local opposition says: "Albrecht is very cautious, but it is clear to me that he is still in favour of the reprocessing plant." However, many opponents of the project feel that at least a battle has been won. Time has been gained which can be used for a national debate on the issue which really ought to have been paramount: the desirability of reprocessing, and of further use of nuclear energy in general, which has been tacitly assumed by German politicians right from the start. □





*"I decided to learn from them": Dr Oku Ampofo (right), director of the Centre for Scientific Research into Plant Medicine, and one of his herbalists*

## When the scientist meets the medicine men

Joseph Hanlon reports from Africa on the increasing respectability of 'primitive' herbal healing

RESEARCH into medicinal plants is now a high priority in Ghana, and it typifies the issues in science there because it is essentially local and yet, at the same time, tied to the developed world.

Herbal medicine researchers use sophisticated analytical chemistry techniques, collaborate with laboratories all over the world and publish in prestige western journals. Yet they rely on the knowledge of traditional healers to solve problems of tropical medicine which have been ignored by a medical science dominated by the developed countries. Herbal medicine conjures up images of witch doctors and 'primitive' cures. In fact, western drugs came almost entirely from plant and fungus extracts until the 1930s. And many drugs, particularly contraceptive pills, still do.

Plant medicine is still the norm in most of the world, and is not being swept aside by synthetic drugs. One reason is that medicinal plants work. Analysis shows they contain many compounds already familiar to pharmaceutical chemists and do have the antibiotic and other properties claimed for them. Indeed, some work for ailments which cannot be effectively treated by modern "scientific" drugs. Herpes zoster (shingles) is an example.

This point has not been lost on multinational pharmaceutical companies. Faced with spiralling research costs, they are paying more attention to traditional medicines and Third World research. Which leads to the sharpest

contradiction for Ghanaian scientists. They are dependent on high technology only available in European and US labs, and collaboration with those labs is crucial. Yet that means the drug companies are likely to get their hands on the results first. Some scientists fear that new drugs discovered in Ghana and other developing countries will be patented by the multinationals and then sold back to developing countries at high prices. The Organisation of African Unity (OAU) has gone so far as to urge secrecy in herbal medicine research to prevent just this.

Publicity about Chinese traditional medicine systems—both acupuncture and herbal—gave traditional healing international respectability, and plant medicine is now being promoted by the World Health Organisation. Before WHO gave its blessing, however, Ghanaian scientists had already turned to this field.

The Faculty of Pharmacy at the University of Science and Technology (UST) in Kumasi has for several years worked primarily on plant medicines. A number of scientists at the University of Ghana and Korle Bu Hospital are also working in this field. Ghana's Centre for Scientific Research into Plant Medicine, founded four years ago, is one of four centres with official OAU backing.

The centre's founder and director is Dr Oku Ampofo. He qualified at Edinburgh in 1939 and returned to his home town, Mampong-Akwapim (now the

site of the centre). "The war was on, and there were no medicines to be had. There were some old people in the district who were treating patients very effectively with herbal medicines. I saw that some of these things worked. Take the case of a woman bleeding after childbirth. In those days, we were far from any hospital to deal with such an emergency. But they had wonderful herbs for post-partum haemorrhage. So I decided to learn from them." Over the years, he studied with a variety of herbalists and compiled a list of more than 300 remedies.

The Ghana government is spending more than £1 million building the new centre, which will have a staff of 20 scientists. Although the building is several years from completion, two scientists have already been hired and sent abroad for further training.

The centre will also contain a clinic both to treat local people and as a research base. The clinic is already operating in temporary buildings. Three doctors and three herbalists work together, treating patients and keeping careful records. One pilot study has already found several herbs that are as effective as the anti-diabetic drug chloropropamide. The centre has its own arboretum to grow useful local plants and it has just been granted a part of a forest reserve in the Accra Plain where it can grow savannah plants from northern Ghana.

The first research step is always to identify as many active compounds as possible. If one is found with a structure akin to a known pharmacologically active substance, then it explains



how the medicine works, and also serves as proof to doubting Western trained doctors who continue to resist plant medicine. Indeed, some of the chemists I spoke to considered their most important role to be convincing doctors that science has an explanation for these herbs. Pharmaceutical chemists are happiest working with compounds close to those they know and understand.

One of Dr Ampofo's plants now being tested at UST is *Cryptolepis sanguinolenta*, which is sold in the local markets, soaked in water and drunk as an anti-malaria drug. It contains a compound very similar to quinoline.

The same plant is also used by herbalists to reduce fever and to cure urinary infections. Microbial tests show that it is a broad spectrum antibiotic, though none of the compounds isolated so far is a known antibiotic. This is an increasingly common occurrence and has led scientists to catalogue the plants in their traditional form, so they can be sold in shops and used by doctors and herbalists who are not familiar with them. Many of the plants are quite common. Using them directly, instead of waiting for compounds to be analysed, synthesised, and converted to tablet form, makes them widely available much more quickly. The Pharmaceutical Society of Ghana now has a policy that where the scientific basis of a remedy can be shown, and where toxicity tests have been conducted, pharmacists should prepare dosages of the crude plant, rather than attempting extracts. Skin ointments, worm expellers and purgatives are now on the market.

## Testing for safety and side effects

But this, too, has its problems. Plants vary widely according to soil and the time of year (and sometimes even the time of day) when they are picked. Some plants can be highly toxic at certain times of the year and not at others. The herbalists know their local plants well, but this doesn't help those without specialist knowledge. Storage also remains a problem. And the method of preparation is often not precise: "Put a little bit into water"—but how much? Dr Ampofo, through his clinical tests, and the UST scientists, by attempting to find the active compounds, are trying to standardise doses.

Traditional medicines, too, have their side effects and some safety testing seems called for. Dr Ampofo notes that "experienced herbalists will warn you that the plant may have a side effect and if it does, to take another preparation to counter it". Also, "most tropical plants contain poisonous alkaloids. In some cases, if one plant is

used for a treatment, then another must be taken as well to eliminate the toxic effect. *Elaeophorbia drupifera* is useful for guinea worm. The herbalist taps the juice and mixes it with palm nut oil, which renders it non-toxic. Otherwise it is poisonous—you purge yourself to death. I know of one herbalist who has an excellent plant for rheumatism, coroinanthe pacchyceras. But it only works in an alcoholic medium—pound it and put dessert-spoonfuls of powder in 20 ounces of gin. Then take one teaspoonful after each meal. If you boil it up instead, it will put you to sleep for two days."

Traditional healers have been ridiculed in the West—and by western-trained Ghanaians—because of the continued failure to find "scientific" justification for their claims. The Ghanaians argue that western scientists are so confident of their own methods that they are unwilling to listen to anyone from a developing country, despite the hundreds of years of careful study of plant medicine by the herbalists. Many Ghanaians consider Western science too reductionist, constantly trying to find a single extract that can be synthesised and put into tablet form. "I don't think the western world knows how fully to analyse a plant yet. There are certain enzymes we know little about and cannot isolate, but which seem to do good work. In any case, isolation of a single compound cannot be the be-all-and-end-all—it may be a synergistic action in the plant that is doing the healing," Ampofo argues.

He criticises scientists who "follow a routine method of analysing all plants; without knowing anything about what they do clinically. They take a long time and then say it's no good. But if you know that plant A stops bleeding, you straight away look for coagulant effects. You don't just go through to get a long list of formulae."

Another reason to listen to herbalists is to find out the method of preparation. "I found a plant which is extremely good for infectious hepatitis —*Bombas boboperzenze*. You cut the bark to pieces, put it in cold water, and put it in the sun for half a day before drinking. I gave it to a professor and he worked on it for a couple of months and then said he couldn't make head or tail of it. I found out that he had boiled it. If the herbalist tells you to do a preparation in a certain way, it doesn't matter if you are a PhD, you must still follow it exactly."

This new scientific approach has already led to promising research on plants for hypertension a problem in village Africa as much as urban England), guinea worm, skin infections, and many other conditions. By looking in their own gardens, Ghanaian scientists have been able to do research

that could not be done elsewhere. They have staked out an area of scientific research and produced world class results which are being published in the top medical and pharmacological journals.

But the going is not easy. Modern analytical chemistry requires sophisticated equipment that Ghana cannot afford. Advanced drug research today is beyond the means of Ghanaian universities. For example, Dr Ivan Addae-Mensahe, a Ghana University chemist, has isolated an amide alkaloid, wisanine, from *Piper guineense*, the Ashanti pepper. The roots and seeds of this plant are used for a wide variety of conditions, including venereal diseases, mumps, and intestinal disorders. It is also used as a sedative. One alkaloid of the plant, nitidine, has been shown to lower blood pressure, so there is considerable interest in the plant as an anti-hypertensive.

## Can Ghana keep the drug companies at bay?

But Addae-Mensahe could only do part of the work. "With my own equipment, I could isolate the compound and tell if it was pure. I could tell the class of compound, its functional groups, and that the structure was close to something I knew. But I needed mass spectrometry and nuclear magnetic resonance—which I didn't have—to tell the detailed structure." His papers on wisanine cite laboratories in Freiburg, London and Cambridge for analytical work. Other work has been done in Australia and Chicago. Much of the work was done as favours by other scientists—his adviser at Cambridge, a scientist he met in Nigeria, and even a contact made by his brother who studied in Germany. The anti-bacteria properties of wisanine were discovered in Germany; its effects on hypertension are being assessed in the US.

Similarly, discovering that *Cryptolepis sanguinolenta* contained a quinoline-like compound was beyond the capability of UST. They had to rely on friends and their own students in universities in Pittsburgh, Bradford and Upsala. The next step is to conduct tests on monkeys, but Ghana has no facilities. As the multinational pharmaceutical companies are now concerned about the rise of strains of malaria resistant to present drugs, they may take over the work.

Where does this leave the Ghanaian scientists? They are concerned that scientific knowledge is far from universal when patents and profits are concerned. Are they, like the cousins on the farms and in the mines, merely supplying raw materials for the multinationals in the developed world? □



# Another cancer scare . . . or is it hypochondria?

WHERE will the search for environmental carcinogens end? A recent article (Commoner, Vithayathil, Dolora, Nair, Madyastha, Cuca, *Science* **201**, 913; 1978) implicates extract of cooked hamburgers; another, (Bruyninckx, Mason, Morse, *Nature* **274**, 606; 1978) oxygen at physiological concentrations. Both were found to be mutagens in the Ames test. Mutagenicity in this case is usually, though not always, associated with carcinogenicity in mammals.

Commoner *et al.* found that ground beef cooked in a home hamburger cooking appliance contained a substance that induced mutations (reversion from histidine dependence to histidine independence) in some strains of *Salmonella typhimurium*. In the *Science* article the authors are properly cautious: "If . . . these mutagens—once purified and tested on laboratory animals—are found to be carcinogens, their apparent concentration in some foods may represent an appreciable risk to certain populations." But the media were less restrained: these cautious claims were blown up in the United States into the Great Hamburger Scare of Fall 1978. Professor Commoner was widely quoted in the press and on TV, and MacDonald hamburgers' stock prospects were re-examined by alert Wall Street analysts.

The Bruyninckx *et al.* findings are even more startling. It has been known for 25 years that hyperbaric oxygen is a mutagen—but mammals are generally not exposed to hyperbaric oxygen. Bruyninckx found that exposure of certain mutants of *S. typhimurium* to 5% O<sub>2</sub>-5% CO<sub>2</sub>-90% N<sub>2</sub> induced as much as a fiftyfold increase in reversion to histidine independence compared to controls kept under anaerobic conditions. In speculating on the significance of their findings, Bruyninckx *et al.* say, "According to Fridovich oxygen toxicity is normally held in check by a balance among rates of formation and destruction of reactive forms of oxygen. This may mean that oxygen mutagenicity is improbable, but not impossible, in normal aerobic mammalian cells; but higher rates of formation of reactive forms of oxygen or lower rates of their destruction . . . could lead to significant rates of mutagenesis along with the molecular pathologies arising from mutation." Thus Bruyninckx *et al.* do not quite say that oxygen, in the form of the O<sub>2</sub><sup>-</sup> radical, may be implicated in carcinogenesis—but others, notably J. Totter, former Director of the Division of Biology and Medicine of the US Atomic Energy Commission, have suggested just this.

Alvin M. Weinberg, director of the Institute for Energy Analysis, turns his attention to the carcinogenicity of oxygen



If oxygen is dangerous, we'll have to stop breathing . . .

These two findings suggest to me that our entire approach to cleansing our environment of carcinogens may be bankrupted by further investigation. Today's environmentalism assumes that environmental agents that do harm, particularly those that cause cancer, are important causes of cancer compared to the natural environment, and also are removable. But these two doctrines have already been shaken by such findings as the presence of nitrosamines in experimental animals fed a normal diet; or for that matter, the existence of the radiation background, not to say of sunlight itself. Professor Commoner's hamburgers are almost unavoidable (though his directions for cooking mutagenic hamburgers may reduce even this exposure). But I would defy even the most ingenious environmental regulatory agency to legislate acceptable levels of oxygen!

Obviously we must get a better idea of how much cancer is attributable to agents that are in principle removable. The oft-quoted assertion that as much as 80% of cancer is caused by environmental agents that are, at least by implication, avoidable, rests on evidence that can hardly be considered compelling, a point recently stressed by Peto (*Nature* **277**, 428; 1979). To be sure, cancer maps show large fluctuations in incidence of specific cancers in different locations; but the fluctuations are much smaller if all cancers in one location are compared with all cancers in another location.

So far regulatory agencies have not raised seriously the question of how much a known carcinogen can add to the unavoidable risk of cancer. If, for example, it turned out that an all-pervasive environmental agent such as oxygen is importantly implicated in cancer, then we may be attacking the one-tenth of the iceberg that shows (the avoidable carcinogens), but ignoring the nine-tenths that is submerged (the unavoidable carcinogens). I offer this speculation to bring home the great difficulties our regulatory agencies face in trying to legislate acceptable levels of exposure.

I should think that before they outlaw MacDonald's hamburgers, or for that matter, before scientists call for a total ban on this or that carcinogen, we await further clarification of the role of all-pervasive agents such as oxygen in the etiology of cancer. We need more scientific understanding much more than we need additional regulation that is based on imperfect and fragmentary evidence.

Where does the scientist's responsibility lie—in publicising the possibility that a commonly used substance might be a carcinogen (Chicken Little), or in withholding publication until he can really assess the risk, say, compared to other carcinogens (Dr Pangloss)? Chicken Little adds to the public's growing environmental hypochondria; Dr Pangloss conceivably might fail to alert the public to a potential danger. Until now Chicken Little has been in fashion, but I hope the pendulum will swing toward Dr Pangloss. The public ought to require of scientific Chicken Littles the same norms of conduct that science itself has imposed: cautious, provable scientific assertions, and a minimum of appeals to the unrefereed public press.

If such an attitude leads to more Panglossism so be it: people will never stop eating hamburgers, let alone reduce their oxygen uptake, no matter what our scientific Chicken Littles and our regulators urge. □



# news and views

## Decaying charm

from D. J. Miller

THE charmed particles are proving a great deal harder to study than the strange particles were. Both the charm quantum number and the strangeness number are conserved by the strong and electromagnetic interactions. This means that charmed or strange particles must undergo weak decays whose rate is determined by two factors; the weak-interaction coupling constant  $G_F$  and the density of allowed final states—the phase-space available. By a happy chance the characteristic lifetimes of strange-particle decays are around  $10^{-11}$  s, so for a particle travelling close to the velocity of light the decay-lengths are around a centimetre or more. This is ideal for bubble-chamber work, where the resolution is approximately half a millimetre, and a wealth of precise data now exists on the lifetime and decay schemes of all the basic strange states. Even the elusive  $\Omega^-$  hyperons are now being studied by the hundred.

But although charmed-particle decay processes are also governed by  $G_F$ , the charmed particles are much heavier than strange particles. This leads to a much greater density of allowed final states for each decay-mode and the predicted lifetime drops to the region of  $10^{-13}$  s, giving decay-lengths of a few hundred micrometres; too short for any normal bubble-chamber to observe. In fact, it is quite difficult even to produce charmed particles in a bubble chamber. Most of our knowledge of them comes from the  $e^+e^-$  colliding-beam machines where  $D$ ,  $D^*$  and other charmed states form a significant part of the total rate, once the charm threshold is passed at about 3.8 GeV

(see *News and Views* 269, 286; 1977). Yet such machines have even worse spatial resolution than a bubble chamber, so the presence of the charmed particles is deduced from observation of their decay products with no hope of measuring the decay-lengths of the parent particles. Nuclear emulsions are, so far, the only detectors which offer a chance to see charmed particle tracks. A lone candidate was reported 3 years ago by an international collaboration, using a stack exposed at Fermilab near Chicago (Burhop *et al.* *Phys. Rev. Lett.* **65B**, 299; 1976). Now a continuation of the same collaboration has reported three new events (Angelini *et al.* *Phys. Rev. Lett.* **80B**, 428; 1979; and Ankara, Brussels, CERN+University College Dublin, University College London, Open University, Pisa, Rome, Turin collaboration preprint, to be published) seen in a stack which was placed just in front of the BEBC hydrogen bubble chamber in the CERN SPS neutrino beam. A few percent of charged current neutrino events are known to give charmed particles in the final state. Without the information from the bubble chamber, and its associated muon detector to establish the charged current, it would take hundreds of man-years to scan a large emulsion stack for a useful number of these events. By spotting likely events in the bubble chamber pictures and extrapolating the tracks back to the emulsion it has been possible to find 101 neutrino interactions in emulsion in less than 2 years. The bubble chamber also gives an opportunity to measure the momenta of tracks leaving the

emulsion and even in some cases to identify the kind of particle which produced a particular track. But the emulsion gives the vital information—charmed tracks are indeed a few hundred micrometres long. Unfortunately, for three of the four events reported so far, the information on the momenta and identities of the secondary particles from the charmed decay has not been good enough to allow the masses and momenta of the charmed particles to be established. There is, however, one event in which a strong case can be made that the decaying particle is the charmed baryon  $\Lambda_c^+$  and, if that is assumed, the momentum can be estimated with some precision. The two figures show tracks from this event. In the emulsion (Fig. 1) a single lightly ionised track travels from the neutrino interaction-star on the left of the picture and splits up into three light tracks after 354  $\mu$ m. These particles all give corresponding tracks in the bubble-chamber picture (Fig. 2), marked a, b and c in the figure. In particular, track c makes an elastic scatter from a hydrogen nucleus in the bubble chamber (see arrow on figure). It has been possible to fit this interaction and identify track c with a high confidence-level, as a proton track. Track a does not match quite as well as the other two with its counterpart in the emulsion—it may have scattered slightly in the steel wall of the bubble chamber.

Ionisation measurements in the emulsion, together with the curvature of the tracks in the bubble chamber, indicate that track a is most probably due to a  $K^-$  and track b to a  $\pi^+$ .



Fig. 1



Fig. 2

Taking the best information on all three tracks the event is consistent with production of a hyperon of mass  $2.3 \text{ GeV}/c^2$  and momentum  $3.74 \text{ GeV}/c$ . It decays to  $K^-\pi^+p$  after living  $7.2 \times 10^{-13} \text{ s}$ . The other three events have similar decay lengths so their decay times must be in the same region.

Because the charmed quark is thought to be much heavier than the everyday  $u$  and  $d$  quarks or the strange  $s$  quark, when a charmed particle decays the process should be simpler than in strange particle decays. To predict the rate for a particular strange decay requires a detailed discussion of the dynamics of two or three-body final states, with special enhancements and even *ad hoc* selection rules. Charm decays, in the current weak interaction theory, should have about the same rate whether the charmed quark is in a charmed baryon or in a charmed meson. The energy released is much bigger than the variations in mass between baryons and mesons, so the quark can decay at its own pace and the final state is rearranged over a comparatively long period afterwards. (This is the same sort of 'parton' argument that is used to explain the deep inelastic interactions of electrons or neutrinos with quarks, quark-pair production in  $e^+e^-$  annihilation or large transverse-momentum jet production in proton-proton collisions). It is thought likely, in view of the number of  $K\pi\pi$  events seen in searches for charm in bubble chamber neutrino events (Baltay *et al.* Columbia-Brookhaven collaboration; *Proceedings of the Oxford Neutrino Conference 1979*, page 198, published by the Rutherford Laboratory) that most of the charmed particles produced by

neutrino interactions are D mesons, not  $\Lambda_c$  hyperons. Some of the other emulsion events are consistent with many-body D decays, so it is encouraging for theorists that the apparent survival times of all events are similar to that of the  $\Lambda_c$  event.

This event is also encouraging on a more fundamental level. As soon as charm was taken seriously it was possible to predict the whole  $SU(4)$  spectrum of charmed mesons and baryons. In the past 5 years many dynamical effects have been explained by the existence of charm, but few members of the charmed spectrum have been firmly established. The  $\Lambda_c$  has been reported before (see for example Cnops *et al.* *Phys. Rev. Lett.* **42**, 197; 1979 and references therein) but each piece of evidence was slender. A well reconstructed  $\Lambda_c$  track is a considerable advance. □

## Bacterial defences against penicillins

from a Correspondent

WE cannot always take a detached view about the outcome of conflicts between one form of life and another. We seek to kill pathogenic bacteria with antibacterial agents—but this onslaught may be resisted by the bacteria, which may produce (or contain) an enzyme that destroys the drug. Penicillins and cephalosporins are among the most effective antibacterial agents and they owe their activity to the presence of the four-membered  $\beta$ -lactam ring. Enzymes that catalyse the hydrolysis of the  $\beta$ -lactam ring—and consequently inactivate the drugs—are called  $\beta$ -lactamases. The increasingly wide occurrence of bacterial  $\beta$ -lactamases has become a source of concern to clini-

cians and a challenge to chemists and biologists. Hence inactivators of  $\beta$ -lactamases are not only tools for enzymologists, they are also potential chemotherapeutic agents. It was against this background that a workshop\* was held recently at the University of Newcastle upon Tyne to discuss the biochemistry of bacterial  $\beta$ -lactamases. There have been significant advances in our understanding of  $\beta$ -lactamase action since the first workshop was held, 4 years ago (see *News and Views* **255**, 526; 1975). Moreover, then we had no effective inactivators of these enzymes; now we have several.

Information about structure, although often laboriously acquired, is particularly significant and enduring. All our knowledge of amino acid sequences in  $\beta$ -lactamases has come from the laboratory of R.P. Ambler (University of Edinburgh) recently—and dramatically—complemented by the work on the nucleotide sequence of a plasmid that encodes a  $\beta$ -lactamase (J. G. Sutcliffe, Harvard University). It is noteworthy that the  $\beta$ -lactamases from *Staphylococcus aureus*, *Bacillus licheniformis*, *Bacillus cereus* ( $\beta$ -lactamase I) and *Escherichia coli* have amino acid sequences that are sufficiently similar to show that they must be classed as homologous proteins. We may thus expect that one mechanism will apply to all these enzymes. By way of contrast, Ambler reported that  $\beta$ -lactamase II from *B. cereus* had so far shown no sequence similarity to the other  $\beta$ -lactamases, and he concluded that this is a strong candidate for an analogous (as opposed to a homologous) enzyme. Moreover,  $\beta$ -lactamase II is exceptional in requiring Zn (II) for activity. S. G. Waley (University of Oxford) (reporting on work carried out in collaboration with H. A. O. Hill and E. P. Abraham) described spectroscopic evidence that the ligands to the essential metal atom were three histidine residues and the enzyme's sole thiol group.

The intermediates in the pathway of synthesis and export of the extracellular  $\beta$ -lactamase of *B. licheniformis* now include species of molecular weight 35,000, 33,000 and 31,000 (J. Lampen, Rutgers University, New Jersey). Structural characterisation of these interesting intermediates is, however, not yet complete.

Information about the three-dimensional structure of  $\beta$ -lactamases is eagerly awaited; J. R. Knox (University of Connecticut) described his progress, both on the  $\beta$ -lactamase from *E. coli*, and on the transpeptidase from *Streptomyces* R 61; which of the structures being studied in several labora-

\*Held on 27–29 April and organised by Professor R. H. Pain and Dr R. Virden (University of Newcastle-upon-Tyne). The workshop was supported by Glaxo Research Ltd.

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tories will be solved first remains to be seen.

Turning from structure to mechanism, one of the highlights of the meeting was J. R. Knowles's (Harvard University) presentation of the first good evidence for an acyl-enzyme intermediate in the enzymic reaction. The hydrolysis of a 7- $\alpha$ -methoxycephem (cefoxitin) by the RTEM  $\beta$ -lactamase from *E. coli* proceeds slowly enough for an intermediate to be isolated at 0 °C; the low molecular weight moiety is covalently bound. When the reaction in solution was monitored by Fourier-transform infrared spectroscopy, the intermediate showed a band at 1753  $\text{cm}^{-1}$ . This is consistent with the intermediate's being an ester, and this, in turn, implicates a serine or threonine residue in the enzyme's action. The importance of serine in  $\beta$ -lactamase I follows from the work of P. G. Sammes (University of Leeds) and Waley who have found that the amino acid residue labelled by an inactivator is serine 44 (serine 70 in Ambler's method of numbering). This is a conserved residue in the sequences referred to above. These results, and those of Knowles, taken together suggest that  $\beta$ -lactamases may now be regarded as 'serine enzymes'. The inactivator used by Sammes and Waley (which was independently discovered by R. S. Pratt, Wesleyan University, Connecticut) was 6- $\beta$ -bromopenicillanic acid, and the labelled enzyme is thought to be a dihydrothiazine. A. F. Coulson (University of Edinburgh) used the sulphone of 6- $\alpha$ -chloropenicillanic acid to inactivate the  $\beta$ -lactamase from *Staphylococcus aureus*: the site labelled is close to (or identical with) that referred to above. The chemistry of the inactivation by these reagents entails acylation followed by fragmentation, according to the views of those using these inactivators, but the structures postulated have yet to be firmly established.

The detailed studies by R. H. Pain and R. Virden (University of Newcastle-upon-Tyne) on the conformation and activity of the  $\beta$ -lactamase from *S. aureus* have led to a three-domain model, with the active site at the interface of two domains. This protein is one of the few for which a partially unfolded form has been well characterised.

Methods of obtaining information about the practical importance of  $\beta$ -lactamases in protecting bacteria against the antibiotics were discussed by G. W. Ross (Glaxo Research, Greenford) and by M. H. Richmond (University of Bristol). Mutants of certain strains of *E. coli* have been obtained lacking the barrier to inflow of antibiotic, and these are helping to throw light on the least-understood

aspects of  $\beta$ -lactamases. These concern the relative positions (in Gram-negative bacteria) of the  $\beta$ -lactamase and the target that the antibiotic interacts with.

As usual, much remains to be done, but significant advances have at last been made in the understanding of these efficient but unwelcome enzymes.

## High energy jets

from M. G. Albrow

In the past few years high energy physicists have progressed beyond the study of the interactions of the strongly interacting particles, the hadrons, to the study of the interactions of their constituents. All known and postulated observable 'elementary particles' fall into three classes (see the review article by Mulvey *Nature* **278**, 403; 1979). These are the leptons (electron, muon, tau and neutrinos), supposedly fundamental particles with half a unit of intrinsic angular momentum (spin) which have no strong interaction coupling, the hadrons (proton, neutron, pions, kaons and so on) which interact strongly, and the field bosons (photon, graviton, intermediate vector bosons) which are the quanta of the force fields acting between the leptons and hadrons. It has become universally accepted that the hadrons are not truly elementary particles but are composite objects. They are probably composed of two types of constituent—quarks, which are very similar to the leptons except that they have a non-zero strong charge, and gluons, which are the field bosons or quanta of the strong force. Thus all matter consists of spin  $\frac{1}{2}$ h particles either with no strong charge (leptons) or with a strong charge (quarks) interacting by exchanging field quanta (bosons).

And yet despite extensive attempts to kick a quark or gluon out of a proton and observe it as an isolated entity, none has succeeded. One can set up a collision experiment that should produce a quark, even to the extent of knowing in which direction it should be produced and with what momentum. In that direction one finds not a single particle but a set of several hadrons moving in roughly the same direction, with a total momentum equal to the momentum the quark should have had. It is just as if the quark produced immediately breaks up into more familiar particles. Such a set of hadrons is called a 'jet', with a jet axis defined as the direction of the vector sum of their momenta. Each member particle has a momentum component parallel to the jet axis and a component transverse to that axis. An observed charac-

teristic of jets is that the average parallel component grows linearly with the total jet energy, while the transverse component remains small, a few hundred MeV/c. Consequently as the total jet energy increases, the angles between the member particles decrease and the jets become more collimated. In the highest energy collisions the jets produced become sufficiently distinct that the multi-jet configuration of the resulting particles appears. Even though in a very high energy collision dozens of particles may be created, it is now postulated that they are all members of a small number (typically 2–4) of these jets. That is to say that a small number of axes can be found such that no hadron has a large transverse momentum component to all the axes. If each jet is taken as signifying the direction of a quark or gluon the description of the collision process takes on a remarkable simplicity.

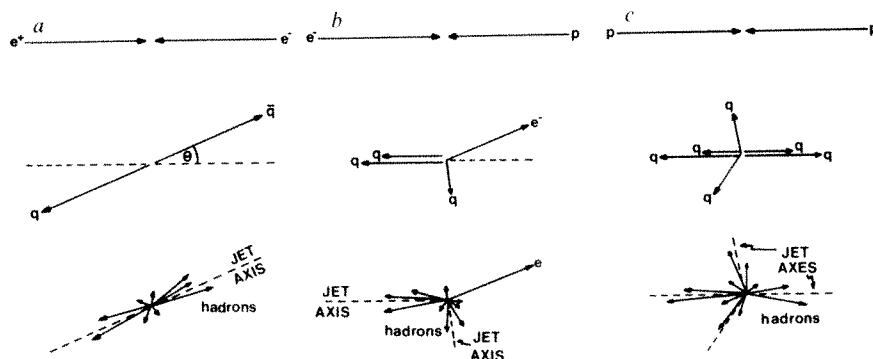
How do these jets appear, in the reference frame in which the total vector momentum of all the particles is zero (the centre-of-mass frame)? In Fig. 1a an electron and a positron collide; they annihilate and all of their energy is converted into hadrons. Typically two colinear jets of hadrons are observed, the axis making an angle  $\theta$  with the collision axis. The angular distribution has a form  $(1 + \cos^2\theta)$ , which is exactly that expected if just two spin  $\frac{1}{2}$ h particles, namely quarks, were produced and then disintegrated into hadrons. Collisions of this type were first seen at SPEAR in Stanford, California in 1975 (Hanson *et al. Phys. Rev. Lett.* **35**, 1609; 1975) and become much more striking at the higher energy now available at DESY in Hamburg (Berger *et al. Phys. Lett.* **78B**, 176; 1978).

In Fig. 1b an electron-proton collision is shown, with the electron emerging intact after suffering a deflection and loss of energy. Again the created hadrons seem to group themselves into a pair of jets which are however not colinear. One of the jets recoils against the deflected electron and its internal structure appears similar to that of the jets produced in  $e^+e^-$  annihilation, suggesting that a quark has been kicked out of the proton and has subsequently disintegrated in the same way. The rest of the hadrons form a second jet pointing along the direction of the incident proton—presumably arising from the remaining proton constituents after one quark has been removed.

A stage further in complexity is the proton-proton collision seen in Fig. 1c. Whenever particles with large transverse momentum relative to the collision axis are observed, they occur in pairs of jets approximately balancing each other in momentum. The natural

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**Fig. 1** *a-c*: Three classes of high energy particle collisions producing jets of hadrons. The initial state, the inferred primary products and the final state hadrons are shown as momentum vectors.

interpretation is that a pair of constituents (one from each proton) has scattered through a large angle.

From these three types of collision process studied at three quite different types of machine ( $e^+e^-$  colliders at SPEAR and DESY, large accelerators at FNAL and the CERN SPS, and the CERN proton Intersecting Storage Rings (ISR) a unified programme of research has now emerged. The first process (Fig. 1*a*) is the ideal way to study the mechanism by which quarks evolve into hadrons, the second (1*b*) can measure the distribution of quarks inside a proton and the third (1*c*) can study the quark-quark scattering process. This threefold attack on the study of the strong interaction was the subject of a Jet Symposium held last year at the Neils Bohr Institute in Copenhagen (*Physica Scripta* **19**, (2) 1979).

Quite how the emerging quarks turn themselves into hadrons is still something of a mystery. Our present understanding of the strong forces between quarks is based on quantum chromodynamics theory (QCD). In this theory the quark-quark force increases as their separation increases, unlike electromagnetic forces where the reverse is true. It may then take an infinite amount of energy to separate a pair of quarks to macroscopic distances. As quarks separate, the energy contained in the increasingly strong field builds up until it is sufficient to 'polarise the vacuum', resulting in a newly created quark-antiquark pair. This process repeats itself until most of their kinetic energy is spent, and the created quarks and antiquarks arrange themselves into hadrons. Unfortunately it is not yet possible to calculate the details of this complicated process. It is still an open question whether free isolated quarks can be produced in sufficiently high energy collisions—it is conjectured, but not yet proved, that they are permanently confined. In that case we may now be exploring the last rung of the ladder down from atoms through nuclei and elementary particles to their most fundamental constituents. □

## Arabic science at Aleppo

from Subir K. Banerjee

FOR historians of Arabic science an important meeting\* took place in Aleppo, recently. The President of this young university Ahmad Y. Hassan, himself a noted historian of technology, has single-handedly provided the impetus for the creation of the world's first major centre devoted to a comprehensive study of all the Arabic sciences, the Institute for the History of Arabic Science at Aleppo University. The institute is also responsible for the publication of the *Journal for the History of Arabic Science*, the first journal of its kind. The term Arabic Science is used in preference to Islamic Science, perhaps to emphasise the idea that we are dealing here with the history of science (and technology) written in the Arabic language which was the language of rational inquiry in the Occident during the period AD 800–1400.

Why should anyone study Arabic science? Before we in the modern West mumble something about its impact on the European Renaissance it is worth recalling what E. S. Kennedy, one of the doyens of studies in Arabic mathematics and astronomy has said, "There need be no laboring of the concealed notion that the providential function of the Arabs was to preserve Greek science against the time when Western Man should awake from his slumbers of the Dark Ages". Taking this view one step further I would like to say that while intrinsically laudable, it is an extremely parochial and ethnocentric view to emphasise in the study of Arabic science only the role of transmission to the Latin West at the cost of minimising the grandeur and

\*The Second International Symposium for the History of Arabic Science was held on 5–12 April at Aleppo, Syria.

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breadth of Arabic science as science *per se*. It is a sobering thought that perhaps only 10% of the total Arabic scientific oeuvre is known to have been translated into Hebrew and Latin. The growth, maturity and decline of Arabic science should be studied primarily as the flowering of human mind during a certain era and for the lessons it can offer to all mankind.

The symposium was successful in driving home this broader viewpoint of Arabic science. One interesting contribution in the sessions on the 'Transmission of Arabic science to the Latin West' was Marie Therésé d'Alverny's discovery of the important influence of Arabic cosmology in the 12th century *Dialogus* of Peter Alphonsi. A highlight was A. I. Sabra's talk in the general session on the place of science and medicine in medieval Islamic civilisation. Over the past few years in private conversations and in short articles Sabra has indicated that he is struggling with the problem of decline of Arabic science and at the symposium he was able to state clearly his new approach to the discussion of this problem: he is concentrating on the careers of the individual scientists of this age, their training, flourishing and activity later in life. In the topical sessions I attended it was most exciting to hear of G. Saliba's identification of the Damascene astronomer al-'Urdi (13th century) as the original author of the planetary model which constituted an alternative to the Ptolemaic model and that 'Urdi's model appears to have been adopted by the more well-known Qutb ad-Din ash-Shirazi. Other interesting new information selected at random is that the Arabs had a magnetic compass for scientific use as early as 13th century and that a 14th century Aleppan astronomer, as-Sarraaj, had invented an exceedingly complex universal astrolabe which, additionally, had the equivalent of a slide rule on its back for complex calculations in spherical astronomy, the trisection of an arbitrary angle and the nature of the lunar surface. □

## Timing time

from P. C. W. Davies

IT has been predicted for 60 years that time runs faster in space than on Earth. Now the prediction has been verified by a direct experiment in which a high-precision clock—an atomic hydrogen maser—was flown into space on board a spacecraft. In a recent issue of *General Relativity and Gravitation*, two Harvard astrophysicists, R. F. C. Vessot and M. W. Levine, report the preliminary results of the experiment,

which took place over the Eastern United States in June 1976.

The effect being verified is caused by the action of gravity on time, which forces clocks (and all natural activity) to run more slowly in the Earth's gravitational field. At high altitudes where gravity is lower clocks speed up. The phenomenon follows directly from the assumption that energy has weight, through a simple argument. When a body is lifted it acquires some extra energy by virtue of the work expended to elevate it. If the energy that drives a clock (for example, elastic energy of a spring or vibrational energy of a balance wheel) also has weight, then additional work must be performed to lift it: crudely speaking, a ticking clock is heavier than an inert one. The extra work expended appears as enhanced internal energy which thereby induces the clock to run more energetically, that is, faster.

The time distortion effect is a completely general one and is really a property of time itself, not merely a mechanical shortcoming of clocks. It is a central prediction of Einstein's general theory of relativity because weighing energy amounts to checking the so-called equivalence principle—that gravity is locally indistinguishable from an acceleration. The effect is minute in the case of the Earth, but in certain astronomical objects such as black holes, it can become enormous.

In 1959 a modest precursor of the Vessot-Levine experiment was performed at Harvard University by R. V. Pound and G. A. Rebka. Very finely tuned gamma rays produced using the Mössbauer effect were shot vertically up a tower 22½ metres high and their frequency at the top compared with that at the base. The tiny shift of only a few parts in  $10^{15}$  could be detected by compensating for it with the Doppler effect, which shifts the frequency of radiation from moving sources. The spacecraft experiment improves on this by probing much greater changes in the Earth's gravity than from the bottom to the top of a tower. The payload was projected 10,000 km into space atop a Scout D rocket launched from Wallops Island, Virginia. At this altitude the time distortion is as much as five parts in  $10^{10}$ , well within the detection capabilities of modern atomic hydrogen masers.

The principle of the experiment was simply to compare the beats of the maser in space with two standard Earthbound masers. In practice there are great complexities involved because other more prosaic effects, such as ionospheric disturbances and Doppler shifting due to the spacecraft motion,

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## More news on SS 433

*from A. C. Fabian*

THE moving emission lines of SS 433 were one of the most exciting astronomical discoveries last year (see *News and Views* 277, 267; 1979). Since the object became visible in the night sky this year many telescopes have been following its behaviour. At the Spring meeting of the American Physical Society in Washington, DC, Bruce Margon (University of California, Los Angeles) reported that the enormous variations in line position are due to the Doppler effect. He and colleagues at the University of California and elsewhere have been prominent in observing and unravelling this bizarre spectrum, and find that all emission lines visible in their spectrographs are split into three components. Those either side of the component at rest wavelength move in the opposite sense and are displaced by an equivalent velocity which varies up to  $+52,000 \text{ km s}^{-1}$  and  $-30,000 \text{ km s}^{-1}$ . The asymmetry is likely to be due to the transverse Doppler effect in emitting matter that is travelling (probably in two diametrically opposite directions) at about  $80,000 \text{ km s}^{-1}$ . The change in velocity represents an acceleration  $\sim 1g$  over several weeks. The overall variation appears to recur on a period of  $160 \pm 3$  days (or some multiple of that). The radial velocity curve passes through a cycle of  $\sim 104 \text{ d}$  which is repeating this year within 2% (including some small but significant variations). The remaining part of the curve should be visible this summer.

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have first to be taken into account. One vital requirement was to obtain accurate information about the spacecraft trajectory, because the time distortion effect is a function of altitude. The accuracy aimed for is about  $\pm 100 \text{ m}$  in position and  $\pm 6 \text{ cm s}^{-1}$  in velocity near apogee, though the authors admit that as yet substantially larger uncertainties exist. Further data reduction will be undertaken to improve the understanding of the trajectory, especially at low altitudes. On the basis of current data, the clock rate distortion predicted by the general theory of relativity is verified to two parts in  $10^4$ .

The principle of equivalence can be checked in other ways, such as the Dicke-Eötvös experiment, which uses a type of balance at a fixed location on the Earth, and is found to be correct

Margon emphasised that this is the first time definite evidence has been found for large scale matter moving towards us at relativistic speeds—even the Paschen lines can be seen in the visible at maximum blueshift. Simply interpreting the radial velocity curve as due to a binary system leads to the picture of two masses of  $2 \times 10^8 M_{\odot}$  orbiting each other just beyond their respective Schwarzschild radii. Perhaps more plausible would be to consider that the emitting matter lies in two jets which are waving around with a period of 160 days. Radio astronomers find evidence for jets in radio galaxies, and relativistic matter squirting out of a galactic nucleus seems to be necessary in order to explain the superluminal expansion in many of these sources. SS 433 may represent a much scaled down galactic version of one of these nuclei. I currently favour a precessing neutron star as the underlying source of energy—Margon has noted that the inferred matter velocity in SS 433 is close to the velocity of escape from such an object. (As an aside it may be noted that SS 433 could be used by an interstellar civilisation to thrust them up to relativistic speeds!)

SS 433 would be visible in binoculars if its position in the galactic plane did not cause significant dimming of its light. Nevertheless, it is within the grasp of many telescopes and must represent one of the most spectacular objects visible. Special relativity can now be demonstrated at significant fractions of the velocity of light on something more than subatomic particles. The emitting mass in SS 433 exceeds  $10^{24} g$ .

to one part in  $10^{12}$ . If the principle is assumed correct, then the Vessot-Levine experiment can be used instead to check that the velocity of light is independent of direction by comparing the one-way and two-way light travel time from the ground to the spacecraft. In fact, the authors comment that there are a whole range of possibilities opened up by the use of high precision clocks aboard spacecraft.

About 70 years ago Einstein and others developed the theory of relativity by appealing to so-called 'Gedanken' experiments in which observers undergo hypothetical experiences involving gravitational fields and different states of motion, while equipped with measuring rods and clocks. In those days spaceflight and super-accurate technology were merely a dream. Now, less than a century later,

some of these fantasy experiments can actually be performed, and the measuring rods and clocks, albeit in technically unrecognisable form, have become a reality. Vessot and Levine point out other experiments which are feasible with current technology using clocks in space. One of these is the measurement of the Lense-Thirring frame-dragging effect, which is a sort of vortex in space in the vicinity of a rotating massive body. Determination of the magnitude of the effect due to the Sun by orbiting clocks near the solar surface in a drag-free satellite, would enable an accurate measurement to be made of the total angular momentum of the Sun, a quantity which is crucial to our understanding of stellar structure. Another possibility is the detection of very low frequency gravity waves which, when washing through the Solar System, cause long-period fluctuations in clock rates. There is no doubt that as technology improves, more and more of the exceedingly minute, though highly significant distortions of space and time predicted by Einstein's theory will come within the scope of direct experimental verification. □

## Micro-ecology

from N. MacDonald

A RECENT review in *Nature* (Bloom 279, 21; 1979) of immunological aspects of parasitism, discusses the methods adopted by parasites to survive within host cells. A full issue of the *Proceedings of the Royal Society* (B204, 1979—arising from a meeting held last year) has recently been devoted to wider aspects of the cell as a habitat for other cells, covering mutualistic interactions as well as parasitism. The evolutionary implications of early symbionts (cells inhabiting cells) which may have become integrated into the structure of more complex cells (present-day mitochondria and chloroplasts for example), have been widely discussed, and are reviewed by F. J. R. Taylor and by J. M. Whatley *et al.* (These and all subsequent references are to papers in the previous reference). The general topic of the micro-ecology of contemporary symbionts, their mechanisms of adaptation to the intracellular environment, and the regulation of their populations by that environment, is probably less widely known.

Various kinds of association of host cell and symbiont can be arranged in order of increasingly close integration. Most symbionts are segregated by en-

closure in a membrane-lined vacuole. Those that are not can typically not be grown outside the host cell. These are presumably candidates for a process in which they lose their separate identity and merge with the host cell, at any rate in cases where the association is mutually advantageous.

This potential loss of identity is not the only way in which the cell as an environment is vastly different from the physical environments of large-scale ecology. Regulation of the population of the symbiont is necessary lest it grows to the extent of bursting the host cell, as well as for restraining the competition of the symbiont for resources needed by the host. In all cases of stable association in which the symbiont is capable of separate existence, the maximum intracellular growth rate of the symbiont population is less than the maximum extracellular growth rate (D. C. Smith). Some of the regulatory mechanisms (as discussed for example by L. Muscatine and R. R. Pool for intracellular algae) are reminiscent of those encountered in animal ecology, at least in so far as one regards the environment of a particular species as including its predators and competitors. The host cell may expel the symbiont, consume it or starve it by competing for a resource. One radically different mechanism, for which there is now some evidence, is the triggering of mitosis in the symbiont by mitosis of the host.

J. W. Moulder discusses the cell as an extreme environment, explicitly examining the partial analogy with physical extreme environments, such as hot springs or salt lakes. Such environments are characterised by low diversity of the species that inhabit them; frequently there is a single dominant species. This species is likely not only to have adapted to the presence of the primary non-biological limiting factor, such as temperature or salinity, but to have become dependent on it. The limiting factors of a host cell are of a more varied and active nature. The cell can exclude or destroy most potential symbionts, so that a single dominant symbiont, which has managed to evade these defences, is typically present. Some kinds of intracellular parasite possess special techniques to gain access to cell interiors, while others simply allow themselves to be engulfed by macrophages, while contriving to avoid subsequent destruction. Enclosure of the symbiont by a vacuole can be thought of as tempering the severity of the extreme intracellular environment.

To give a final twist to the discussion of this complex and intriguing kind of biological association, M. H. Richmond discusses the bacterial cell as a habitat for extra-chromosomal DNA frag-

ments, such as plasmids, which can specify resistance to antibiotics or the ability to metabolise novel substrates. In this context plasmids can be regarded as molecular symbionts whose effect is to allow the bacteria which carry them to penetrate otherwise inhospitable habitats. □



## A hundred years ago

THE first public act passed by the U.S. Congress during the present session, was one making an appropriation of 200,000 dollars for the construction, under the direction of the Secretary of the Treasury, for the National Board of Health of a vessel provided with suitable refrigerating apparatus, for the purpose of determining the possibility of destroying the yellow fever infection by intense cold. The act first introduced had special reference to the apparatus of Prof. Gamgee, but as passed it is within the power of the Secretary to select any device that will, in the opinion of the National Board of Health, best answer its purpose.

THE *Colonies and India* furnishes some interesting particulars respecting the so-called "vegetable ivory," which is now so much used as a substitute for ivory. The vegetable ivory nut is the produce of a species of palm found wild in South America and Africa. Inside the hard shell is the white kernel, which being softer than ivory and easily carved, as well as readily dyed, and being less brittle than bone, is largely used in making buttons, &c. The unripe fruit consists of a green shell, containing a watery fluid, which, as the nut ripens, gradually thickens until it becomes a pulpy mass, and eventually hardens into solid matter. The water, though bitter to the taste, is wholesome, and often renders invaluable service to travellers, who cannot otherwise obtain water to drink. The tree (*Phytelephas macrocarpa*) on which the fruit grows is unlike an ordinary palm, having little or no stem and drooping downwards, especially when the weak branches are overweighted by the six or seven bunches of nuts, each containing six or seven seeds, inclosed in thick heavy shells and outer sheath, and weighing altogether from 20 to 24 lbs.

EXTRAORDINARY finds of gold have lately occurred in the gold-fields of Dutch and French Guiana and are causing great excitement.

From *Nature* 20, 22 May, 88, 89; 1879.

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# review article

## Virological evidence for the success of the smallpox eradication programme

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*Smallpox has prevailed throughout the populated world during the last four centuries but the World Health Organization's eradication campaign has now apparently interrupted transmission of the disease in the human population. The risk of natural re-introduction of the disease is negligible.*

TEN years ago the worldwide incidence of smallpox was estimated to be about 10 million cases annually but since October 1977, when a case of smallpox was recorded in Somalia, there has been no naturally occurring smallpox reported throughout the world. Hopes that the case in Somalia would be the last instance of smallpox in the world were destroyed by the outbreak of smallpox in Birmingham, UK in August 1978. This caused considerable concern among the scientific community as well as the public because laboratory virus stocks appear to have been the source of infection. Although the focus of attention has been on measures to minimise the potential danger of variola virus stocks in laboratories, it is equally important for the world scientific community to examine whether variola virus has really become extinct in the human population and if so, what future surveillance and research on orthopoxviruses are required to ensure the permanent status of this phenomenon. I attempt in this article to present a summary of scientific data and review these problems.

### The origin of smallpox

Ancient records in China and India imply that smallpox, easily recognised by the descriptions of its typical rash, high death rate and extensive transmissibility, was present in the eastern part of the Asian continent long before the Christian era. There are also records from that time of attempts to immunise susceptible persons with extracts of smallpox lesions (variola).

As man is the sole host of variola virus and no animal reservoir exists, continuous transmission of variola virus in the human population required aggregates of susceptible individuals such as first occurred about 5,000–6,000 yr ago when agricultural development made it possible to support populations of more than 500 living together in one place (F. Fenner, personal communication). The disease apparently spread in all directions from the eastern part of Asia along with population movements due to trade, religious and political conflicts and exploration. The disease first appeared in Asia Minor in the early Christian era and around the same period southern Europe was infected. Smallpox reached Japan in the eighth century, the Americas in the sixteenth century, Siberia in the seventeenth century and Australia in the eighteenth century. Its devastating effect when first introduced into a new population has been well described. The disease probably originated from one place—eastern Asia—roughly 5,000 yr ago and has prevailed over the past four centuries throughout the populated world.

A finding inconsistent with this theory is that Rameses V of Egypt died of an acute disease in 1,100 BC which left pock-like

lesions on his mummified face. Electron microscopic examination of the pock lesions on this mummy might shed light on the diagnosis of the disease and the World Health Organization (WHO) and the Egyptian government are discussing this possibility. If Rameses V's illness was smallpox, this means that smallpox was already present in northern Africa at that time. The Roman, Egyptian, Babylonian, Assyrian and Persian civilisations have left considerable historical records, but there is no evidence indicating a smallpox epidemic in these areas around that time. Two hypotheses can be proposed; the disease may have been introduced from the East (commercial trade between Egypt and Asia was well established as far back as 1,100 BC) and, leaving no recognisable records of a smallpox epidemic, may have died out spontaneously; or, the source of Rameses V's disease could have been the African continent, perhaps Central Africa with which Egypt might have had communications along the River Nile. However, there is no way of determining which hypothesis is correct.

### Prevalence of smallpox

At the end of the eighteenth century, Edward Jenner showed that material extracted from the lesions of cowpox would immunise against smallpox, and could replace variolation. During the following 150 years Jenner's 'vaccine' was gradually accepted by health services all over the world. However, over the years the virus strains used for vaccine production did not remain identical to those used by Jenner. By the 1950s, the existence of an effective vaccine and its coordinated use in vaccination programmes had resulted in the interruption of indigenous smallpox transmission in many countries in the temperate zones. Smallpox was, however, still prevalent in many of the tropical countries of Africa, South America and Asia. One reason for this was that the smallpox vaccine rapidly lost potency when exposed to tropical climates.

In the early 1950s a method of freeze-drying smallpox vaccine, so that it retained its potency for at least 4 weeks at 37°C, was adapted to large-scale production<sup>1</sup>. This freeze-dried vaccine, stable in tropical climates without refrigeration, greatly enhanced the prospects for smallpox eradication.

In 1967 the WHO initiated an intensified global smallpox eradication programme. At that time, smallpox was endemic in 33 countries and cases were reported by 44 countries (Fig. 1). The campaign involved a massive investment of manpower, vaccine, technology and managerial skills. The total cash input from international assistance during the past 12 years is estimated to be about \$100 million. The essential strategy of the

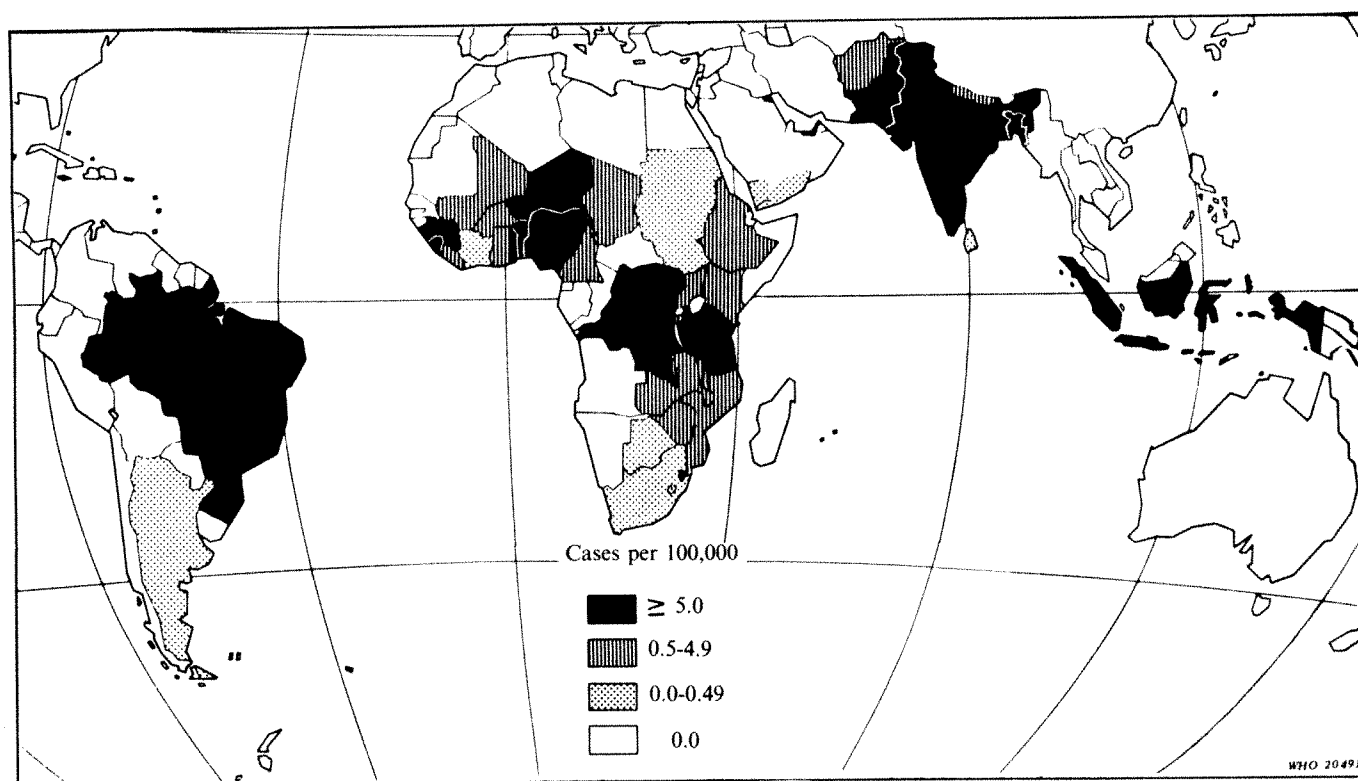


Fig. 1 Smallpox cases per 100,000 inhabitants in 1967.

programme was epidemiological surveillance combined with prompt containment of outbreaks detected<sup>2</sup>.

Among milestones in the progress of the smallpox eradication programme have been the recording of the last cases in South America and West and Central Africa in 1971; in Indonesia in 1972; in southern Africa in 1973 and in the Asian subcontinent in 1975. Since 1976, the endemic foci were confined to the Horn of Africa. The last known case of smallpox of natural origin was detected in Merka town, southern Somalia in October 1977 (Fig. 2).

### Certification of interruption of smallpox transmission

Do any foci of smallpox remain hidden in densely populated slum areas, remote tropical rain forests or isolated deserts? Past suspicions of this had some justification. A pockmark survey in Nigeria in 1971 revealed that the completeness of smallpox case reporting before the campaign began was only 1% in rural areas and 8% even in urban areas. In India, in 1973, when an active search for hidden foci was first carried out, it was revealed that less than 5% of all cases were reported in the entire country.

As the smallpox eradication campaign progressed, however, the efficacy of smallpox surveillance was considerably increased. National eradication programmes developed search operations for hidden foci even in the most remote areas, particularly when smallpox incidence in these countries became very low. However, there were a few exceptions. In Botswana an outbreak was detected six months after the last case was thought to have been found, and an outbreak in Indonesia followed a gap of 8 months in which no case was reported. Hence it was decided by the WHO Expert Committee for Smallpox Eradication in 1972 that active surveillance should be continued for at least 2 years after the last known case before eradication could be declared. The kind of major surveillance measures undertaken during this 2-year period in each of the countries concerned are illustrated by the following examples.

In India and Bangladesh, massive house-to-house searches were undertaken in the 2 years following the detection of the last

cases. On combining the data for the two countries it is found that an average of 98.2% of the 726,811 existing towns and villages were visited during each of the searches. Three such searches were conducted in India and eight in Bangladesh. No cases were found. Pockmark surveys were carried out in 28 countries of West, Central and southern Africa from 1975 to 1978. Of the total estimated population over 4% (9,059,119) were seen. No pockmarked person was found among children born after the year of the last known case in each country. In Ethiopia and Somalia, where the last endemic foci were detected during 1976 and 1977, the latest assessment results illustrate the intensity of the search for hidden foci; over 75% of the inhabitants had met search workers who enquired about smallpox rumours, over 75% knew about the reward which was offered to them if they found a smallpox case (Fig. 3), and over 70% knew where to report such a case.

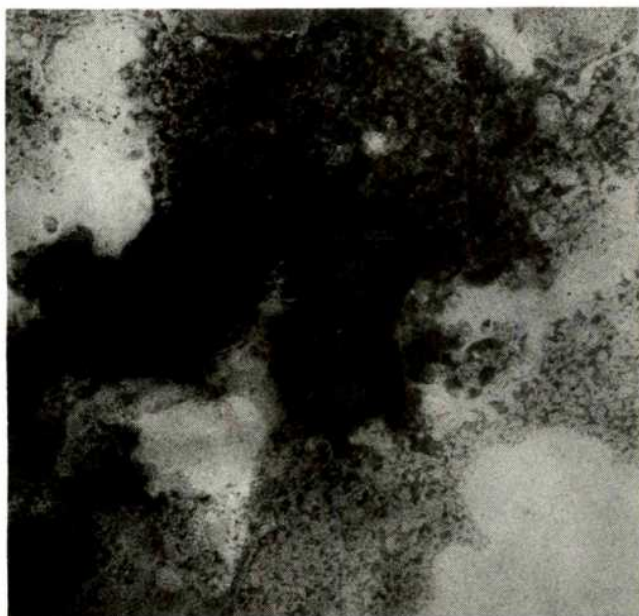
The adequacy of all these activities has been verified independently during visits by an international group of experts (the International Commission for the Certification of Smallpox Eradication) convened by the WHO for certification purposes. Seventy-nine countries have been identified as either recently endemic for smallpox or exposed to the risk that an imported case of smallpox might establish endemicity. Of these, 64 countries have already been certified as free of smallpox and the remaining 15 countries are preparing for certification by the end of 1979 (Fig. 4).

### Laboratory investigation of collected specimens

From 1973 to 1978, 11,249 specimens were collected from 51 countries—all situated in the tropical zone of Africa and Asia. All the specimens were tested by the WHO Collaborating Centres in Atlanta and Moscow with at least three standard methods—electron microscopic examination, virus isolation on chorioallantois of embryonated eggs and precipitation in gel testing. As the incidence of smallpox fell, so the hunt for residual foci intensified so that in 1977 and 1978, 8,507 specimens were tested.

Over 650 strains of variola viruses have been isolated, all of





**Fig. 2** Variola virus particles (variola minor) from a specimen taken from the last reported case, 26 October 1977.

which conformed with our previous understanding of the characteristics of this virus. Also, vaccinia virus (smallpox vaccine virus) has been isolated on several occasions, indicating that either the suspect patient had generalised vaccinia, or the specimens were contaminated with vaccinia virus. Monkeypox has been found in 36 sporadic cases of zoonosis in West and Central Africa (see below). And on one occasion, a poxvirus termed 'Lenny virus' was isolated from a person with rash and fever who died in eastern Nigeria in 1969<sup>3</sup>. This virus showed a mixture of the characteristics of variola and vaccinia virus, indicating a possible hybridisation of variola and vaccinia viruses during double infection in man. There was no evidence that this virus was transmitted among the inhabitants in the area.

### Mild and severe smallpox

Laboratory examination of variola viruses isolated during the eradication campaign has shed light on the presence of different subspecies of variola virus. Since the report of the presence of mild smallpox 'Amaas' in southern Africa early this century, it has been recognised that there are two strains of variola virus, one, variola minor, causing fatality rates of less than 1% and the other, variola major, causing fatality rates of 20 to 40% among unvaccinated patients. The epidemiological investigation of 36,069 cases, including 2,444 deaths, during the past two decades indicated that fatality rates vary considerably according to the geographical areas; 15–36% on the Asian subcontinent, 8–13% in Africa and Indonesia, and less than 1% in South America<sup>4</sup>. Laboratory studies of more than 200 isolates obtained from these regions indicated that there were at least three sub-groups (A, B and C) in terms of haemadsorption tests on human embryo cell cultures infected by such isolates at 40 °C (ref. 5). Group A was dominant on the Asian sub-continent where the case fatality rate was highest, group B on the African continent where the case fatality rate was moderate, and group C in South America where it was the lowest. Thus, the conventional classification into variola major and minor appears to be inadequate; instead there might be a spectrum of variola virus strains varying considerably in their pathogenicity to man. Although this might indicate that the selection of a less pathogenic variola virus had occurred during long-established endemicity, it was essential for the global smallpox eradication

programme to exterminate all variola virus, regardless of its pathogenicity to man.

### The possibility of variola virus in animals

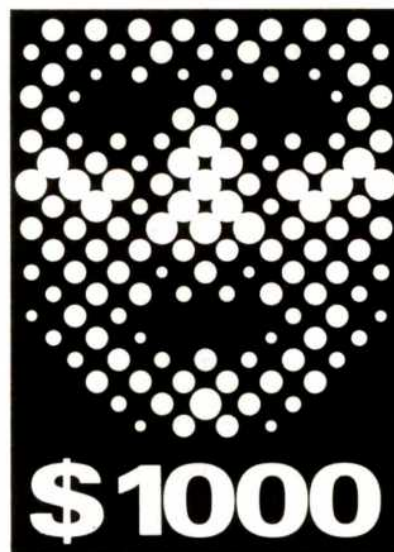
In the literature from 1767 to 1949 there are seven references to a smallpox-like disease in non-human primates<sup>6</sup>. Except for the one in Indonesia, which occurred in the Jakarta Zoo in 1949, there was no valid evidence that any of these was true smallpox. Between 1966 and 1970, 7,497 blood and tissue specimens collected from mammals in West Africa were examined for various viruses<sup>7</sup>. Of the specimens, 104 were from non-human primates and 5,517 were from rodents. Among 83 virus isolates obtained there was only a single pox virus (gerbilpox) and it was distinguishable from variola virus. From 1970 to 1975, 1,024 sera were collected from non-human primates from tropical Asia without obtaining any evidence of poxvirus infection<sup>8</sup>.

More important are the epidemiological findings. Notably, in the Philippines and Central America, where smallpox has been eradicated for several decades, it has never returned spontaneously, although in these areas non-human primates are plentiful. Also, during the last 10 years of the smallpox eradication campaign, comprehensive epidemiological investigations were conducted on more than 50,000 outbreaks in South America, Africa and tropical Asia. None of the sources of infection could be traced back to an animal origin.

### Orthopoxviruses in animals

Variola virus is a member of the genus *Orthopoxvirus*. Six important viruses in this group are presented in Table 1. All these viruses have close antigenic relationships. Vaccinia, cowpox and camelpox viruses lack continuous transmissibility in man, but the situation with monkeypox virus and whitepox virus deserves further comment.

**Monkeypox virus:** In 1958 a poxvirus was isolated from an outbreak of pox disease in a captive monkey colony in a laboratory in Copenhagen<sup>9</sup>. The virus was called monkeypox virus. The WHO, in surveys on 27 laboratories from 11 countries conducted in 1968 and 51 laboratories from 25 countries in 1970, came up with 10 previous monkeypox outbreaks in laboratories. In one instance monkeypox virus was isolated from a giant ant-eater which temporarily shared a cage with an infected monkey. No monkeypox infection has been reported in laboratories since 1969. No human infections have been associated with any of these outbreaks but the vaccination status of those in contact with the monkeys is not known.



**Fig. 3** "The World Health Organization offers \$1,000 to the first person reporting an active smallpox case resulting from human-to-human transmission and confirmed by laboratory tests. Valid until global eradication is certified."



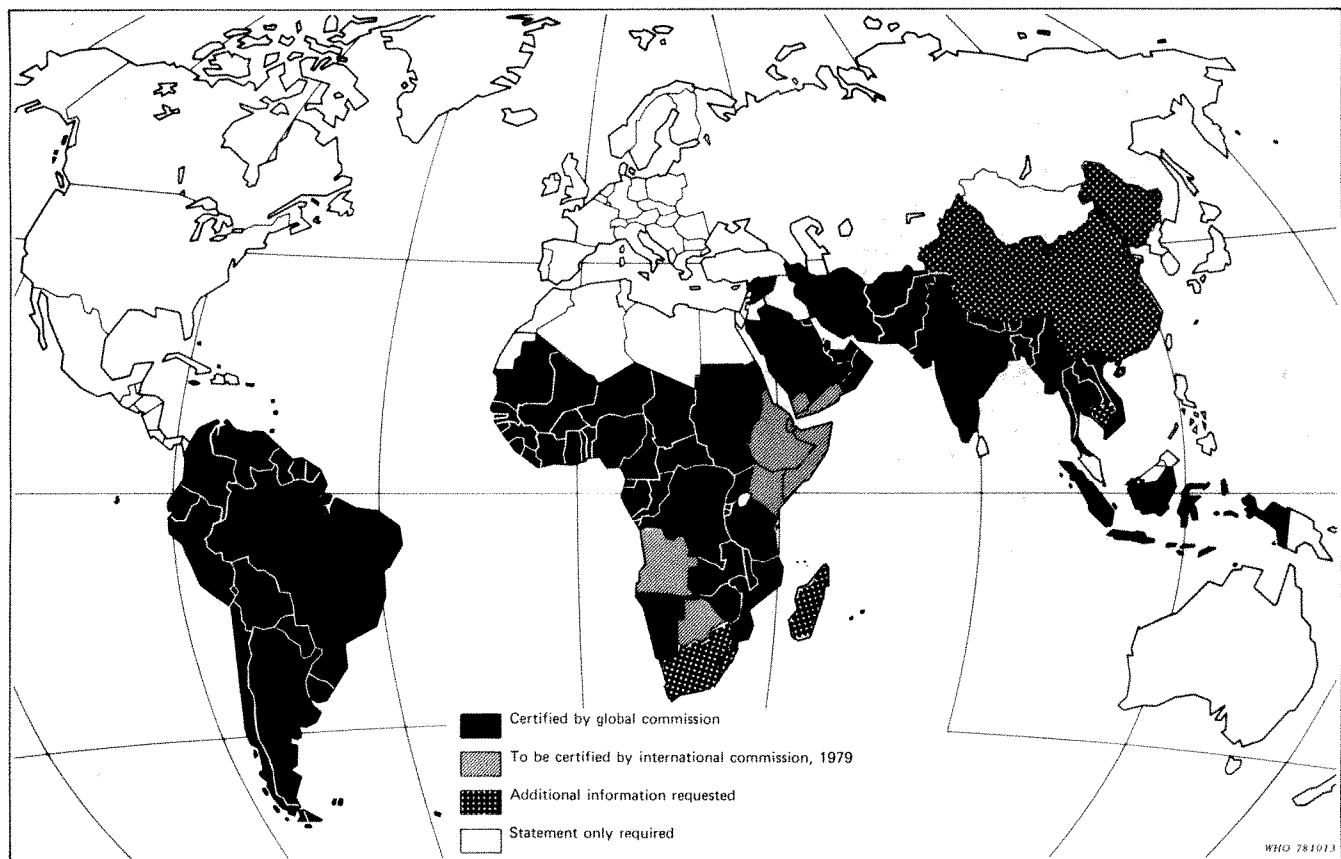


Fig. 4 Global certification of smallpox eradication by the end of 1979.

Monkeypox has not been reported in wild monkeys. The virus was considered a laboratory curiosity until 1970 when the first case of human disease was discovered in remote tropical rain forest of Equateur Region, Zaire<sup>10,11</sup>. From 1970 to 1978, 36 cases of human monkeypox were reported from West and Central Africa. In Zaire, 27 cases have occurred, in Liberia, four, in Nigeria, three, and in the Ivory Coast and Sierra Leone, one each. All the cases have clinically resembled smallpox. Six persons have died, a mortality rate (16%) similar to smallpox in West and Central Africa. Only four patients had a vaccination scar. Most cases occurred in the zone of tropical rain forests where people are hunters and have frequent close contact with a variety of domestic and wild animals (Fig. 5).

There has been no evidence of person-to-person transmission except in two families where second cases occurred 8 and 12 days after the first. These cases suggest secondary transmission unless separate exposures to a common source of infection occurred. Assuming secondary transmission did occur, the potential for interhuman spread of monkeypox can be estimated from the number of susceptible family and community members (those without a smallpox vaccination scar) who had close contact with patients having active disease. Of 56 susceptible contacts, only two (3.6%) came down with the disease. This secondary attack rate is 10 times lower than the transmission rate of 35% for smallpox estimated in West Africa. Tertiary spread of human monkeypox has never been reported.

Special vaccination scar and facial pockmark surveys were carried out in 1975 in populations living near where human monkeypox cases had occurred 4 to 5 years previously in the Ivory Coast, Liberia, Nigeria and Sierra Leone. A relatively low immunity level was found in younger age groups. There were no vaccination scars in 57% of 2,125 children aged 0–4 yr and 29% of 8,047 school-age children, indicating their probable susceptibility to monkeypox infection. No evidence of monkeypox (or smallpox) was found.

The source of human monkeypox infections is as yet unknown. Recent serological studies conducted in the immediate areas where human monkeypox cases occurred have shown a 23% (50/125) prevalence of poxvirus-neutralising antibodies in non-human primates<sup>12</sup>. Of these, three sera showed monkeypox-specific antibody<sup>13</sup>. No human monkeypox has been reported from South America or Asia. Little is known about its natural history, possible animal reservoir or pattern of transmission. Thus, although monkeypox does not appear to frustrate the achievement of smallpox eradication, further research on monkeypox in West and Central Africa with special emphasis on Zaire is warranted. It will be supported by the WHO, for whom the clinical resemblance of the disease to smallpox is one of the problems encountered in its continuing surveillance for smallpox.

**Whitepox virus:** Although attempts to isolate monkeypox virus from animals captured near human monkeypox cases have failed, four whitepox virus isolates have been obtained during laboratory examination from 1970 to 1978. These wild whitepox strains came from the kidney tissue of one chimpanzee, one wild monkey and two rodents all captured in Equateur Region of Zaire<sup>8</sup>. Before this, two strains of whitepox virus had been isolated from routine monkey kidney cell cultures in 1964–65 in another laboratory. The monkeys had come from Malaysia. So far laboratory tests have been unable to determine the nature of these isolates or to distinguish them from variola virus. Note, however, that there has been no evidence of human infection by the virus in the areas where animals for specimen collection were captured.

In 1978, a laboratory reported that cloned variants of some monkeypox virus strains showed the characteristics of whitepox virus and that in hamsters inoculated with a monkeypox virus strain, monkeypox virus was isolated at an early stage of infection but a few whitepox virus isolates were made at a late stage of infection<sup>14,15</sup>. However, the experiences of several other

**Table 1** Comparison of some orthopoxviruses

Characteristics	Variola	Whitepox	Monkeypox	Vaccinia	Cowpox	Camelpox
Isolated from	Man	Ape, monkey rodent	Man, monkey anteater	For vaccine production, origin unknown	Man, cow, large felines	Camel
Pocks on chorioallantois of chick embryo	Small, white	Small, white	Small, pink	Large, white to grey	Haemorrhagic	Small, white
Ceiling temperature on chorioallantois of chick embryo (°C)	37.5–38.5	38.5	39	41	39.5	38.5
Growth in rabbit skin	—	—	++	+ or ++	++	+
Pathogenesis for baby mice	Low	Low	High	High	High	Low
Antigens specific to:						
Vaccinia	—	—	—	+	+	+
Variola	+	+	—	+	?	+
Monkeypox	—	—	+	—	?	—
Polypeptide pattern	Character of variola	Character of variola	Character of monkeypox	Character of vaccinia	Character of cowpox	?
Thymidine kinase sensitivity	+	+	—	—	—	—

investigators have produced conflicting results, namely that white pock variants of monkeypox have not been similar to whitepox virus. Further experiments to clarify these two sets of different observations are now under way in two WHO Collaborating Centres.

Although laboratory studies on whitepox virus have proved somewhat confusing, epidemiological evidence certainly suggests that whitepox virus must differ from variola virus in some way because: (1) no human infections with a variola-like virus have occurred in Malaysia during the last 12 years or in Zaire during the 7-year period since the last smallpox cases were recorded; and (2) it can be presumed that the surveillance system, particularly in Zaire, is sensitive enough to have detected any infection with a variola-like virus as shown by the capability to detect human monkeypox. Nevertheless, this virus merits continued laboratory and field investigations.

### Mutation of poxviruses to variola virus

History suggests that smallpox arose in one area and spread across the world from the initial focus. Although other orthopoxviruses appear to be widespread in nature, there is no evidence of any other 'spontaneous' appearance of smallpox. This is strong evidence against the hypothesis that other poxviruses might give rise to variola virus by mutation.

### Persistence of virus in convalescence and on fomites

Variola virus can be recovered from the urine or nasopharynx of infected patients for not more than 25 days after the onset of the rash<sup>16</sup>. This finding is consistent with epidemiological experience that recovered patients are no longer infectious.

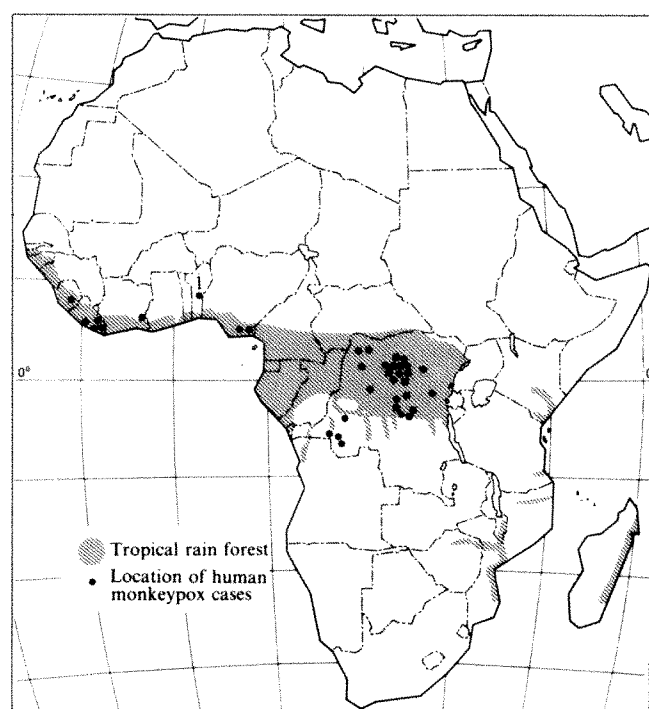
The possibility of smallpox scabs remaining in the houses of patients in previously endemic areas has caused concern, as in 1968 it was reported that scabs kept at room temperature on a shelf in a laboratory in a temperate zone for over 13 years contained viable virus<sup>17</sup>. More recent experiments, however, measuring virus decay in tropical conditions, indicate that virus concentration in scabs may decrease to a non-infective level within three weeks<sup>18</sup>.

The practice of variolation persisted until very recently in some remote tropical areas of Africa and Asia. The last known use of variolation was in Ethiopia in 1976. Samples of variolator's stocks have been collected from Afghanistan, Ethiopia and Pakistan during the past 10 years. Of 21 specimens, 17 did not grow variola virus despite the fact that several specimens showed numerous poxvirus particles on electron microscopy.

Four specimens, all from Afghanistan were positive for viable virus when tested 4–9 months after the variolators had collected their material, but Afghanistan has maintained freedom from smallpox for 5 years; the last case was recorded in 1973. In Ethiopia, once smallpox outbreaks stopped, the practice of variolation also ceased.

### Conclusions

Current evidence from field and laboratory investigations indicates convincingly that when the interruption of smallpox transmission in the human population has been confirmed throughout the world, the risk of re-introduction of the disease will be negligible. This assessment may logically lead to the universal termination of routine smallpox vaccination programmes. This, however, should be accompanied by continuing studies and vigilance. The surveillance of orthopoxvirus



**Fig. 5** Location of human monkeypox cases 1970–78. (1, Patient reported to a hospital in Benin, but developed pockmarks in western Nigeria.)

infections in animals and man as well as laboratory studies of orthopoxviruses should continue and all necessary steps should be taken to minimise the potential danger of live variola virus stocks. To this end, the first meeting of the Global Commission for the Certification of Smallpox Eradication was convened by the WHO in December 1978 and it made comprehensive recommendations in relation to surveillance and research after certification of smallpox eradication, which is expected to take place by the end of 1979.

I thank Professor Keith Dumbell, Dr D. A. Henderson and John Wickett who gave advice, and all colleagues in many countries who provided substantial data for the assessment of the smallpox eradication programme.

*Note added in proof:* Since submission of this article smallpox eradication has been certified in six more countries: Angola, Botswana, Iraq, Lesotho, South Africa and Swaziland. Thus there remain only nine countries which are preparing for certification before the end of 1979.

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## articles

### Sm–Nd dating of Onverwacht Group Volcanics, southern Africa

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*Sm and Nd were unaffected by alteration of the Onverwacht lavas permitting for the first time a precise Sm–Nd date ( $3,540 \pm 30$  Myr) to be obtained for this major igneous event.*

THE Onverwacht Group Volcanics, occurring in Swaziland and the Transvaal of South Africa, are probably the most extensively studied Archaean greenstone belt volcanics<sup>1–7</sup>. Ultramafic volcanic rocks were first well documented from the Onverwacht<sup>1–3</sup> and have been generally termed komatiites<sup>4</sup>. Much of the vigorous debate concerning the nature of the Earth’s first stable crust has centred on comparisons of structural, metamorphic and geochemical features of the Onverwacht Group and the Ancient Gneiss Complex<sup>1,5–7</sup>, the oldest element of the granitic terrain surrounding the Swaziland Supergroup.

The Onverwacht Group forms the basal part of the Barberton greenstone belt and together with the overlying sedimentary Fig Tree and Moodies Groups constitutes the Swaziland Supergroup. The Onverwacht Group itself consists of two distinct volcanic units<sup>1</sup>. One of these, the lower ultramafic unit (LUU), is separated from the other, the mafic-to-felsic unit (MFU) by a persistent sedimentary band termed the Middle Marker Horizon. Each unit is subdivided into three formations which from base to top are the Sandspruit, Theespruit and Komati and the Hoogenoeg, Kromberg and Swartkoppie Formations<sup>1</sup>. The

LUU consists mainly of peridotitic and basaltic komatiites with subordinate basic and acid tuffs, and small, generally concordant, penecontemporaneous, felsic porphyry intrusions<sup>2</sup>. The MFU is of less mafic character and has a greater abundance of chemical sediments<sup>3</sup>.

Despite the considerable geological importance of the Onverwacht Group, geochronological studies have been unsuccessful in either precisely defining the time of the volcanism or establishing the temporal relationship between the Swaziland Supergroup and the Ancient Gneiss Complex. Whole rock Rb–Sr studies<sup>8</sup> of the latter indicate repetitive metamorphism and Sr isotope rehomogenisation for ~200 Myr after initial formation of the gneisses ~3,300 Myr ago ( $^{87}\text{Rb}/^{87}\text{Sr} = 1.42 \times 10^{-11} \text{ yr}^{-1}$ ). The initial  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of  $0.7006 \pm 24$  (all quoted errors are at the 95% confidence level ( $2\sigma_m$ )) for those gneisses at 3,300 Myr when compared with probable mantle values during the Archaean constrain a single-stage crustal prehistory of their parent materials to a period of no more than 100–150 Myr.

Greenschist facies metamorphism which pervades the Swaziland Supergroup<sup>1,9</sup> has perturbed the Rb–Sr systematics such that they now give conflicting impressions as to the age of volcanism. For example, whole-rock Rb–Sr data<sup>10</sup> from the felsic volcanics suggest an age of  $2,570 \pm 40$  Myr, whereas Rb–Sr results for whole rock mafic volcanics provide no useful geochronological information<sup>10,11</sup>. The Rb–Sr ages obtained on sediments of the Swaziland Supergroup are  $3,280 \pm 70$  Myr for



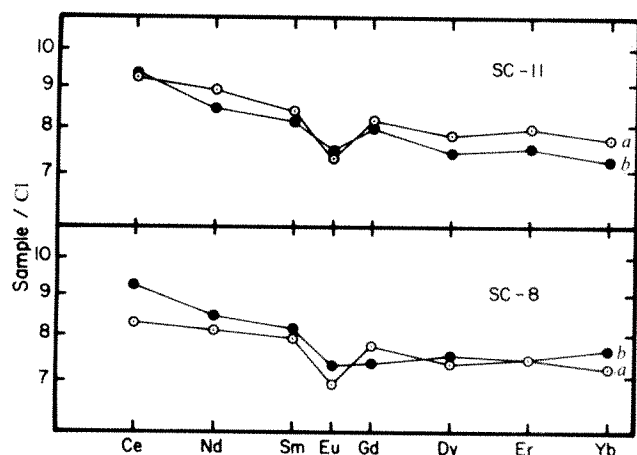


Fig. 1 C1-chondrite normalised<sup>23</sup> abundance patterns for two pillow margin(a)–interior(b) pairs.

the Middle Marker Horizon<sup>12</sup> and  $2,920 \pm 20$  Myr for the Fig Tree Group<sup>13</sup>. These results provide minimum times for the deposition of the sediments and indicate that the Rb–Sr data for the felsic volcanics reflect metamorphic resetting at 2,570 Myr (ref. 10). The U–Pb<sup>14</sup> results obtained on zircon and sulphide separates from felsic volcanics of the MFU yield a slightly older age than the Rb–Sr data at  $\sim 3,310$  Myr. Furthermore, an internal isochron obtained from mineral density separates of a basaltic komatiite from the Komati Formation yields a Rb–Sr age of  $3,430 \pm 200$  Myr and a well defined initial  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of  $0.70048 \pm 5$  (ref. 11).

Despite considerable effort, adequate geochronological data have not yet been obtained on the Onverwacht volcanics. We present here the first definitive age for the Onverwacht volcanism obtained from Sm–Nd analyses of whole-rock samples.

## REE abundances

The difficulty of obtaining reliable ages from the Onverwacht volcanics using a parent–daughter system which involves an alkali and an alkaline earth element is not surprising in view of the evidence for element mobility obtained from volcanic rocks which have suffered low-grade alteration<sup>15–22</sup>. Both Condie *et al.*<sup>22</sup> and H.S.S. and A.J.E.<sup>20</sup> have investigated the effects of metamorphism on the chemistry of Onverwacht volcanics. Whereas H.S.S. and A.J.E.<sup>20</sup> have made detailed investigations of pillow lava margins and interiors, Condie *et al.*<sup>22</sup> have studied the variations within individual massive flows. Both studies, however, indicate variable fractionation of Rb/Sr during alteration and also demonstrate labile behaviour for K, Na, Ba, S, Cu and Zn, for example. Condie *et al.*<sup>22</sup> have further suggested that the light rare-earth elements (REE) have migrated relative to the heavy REE, although the evidence for such a conclusion is sparse.

Table 1 REE abundances for two LUU pillow lavas

	SC-8		SC-11	
	Margin (a)	Interior (b)	Margin (a)	Interior (b)
Ce	10.3	11.5	11.5	11.6
Nd	7.3	7.6	8.0	7.6
Sm	2.21	2.27	2.34	2.27
Eu	0.72	0.76	0.76	0.78
Gd	2.74	2.60	2.90	2.83
Dy	3.13	3.21	3.34	3.16
Er	2.02	2.02	2.17	2.05
Yb	1.88	1.99	2.01	1.89

To further assess the possibility of differential behaviour of REE during the alteration of Onverwacht samples, REE abundances have been determined using precise isotope dilution techniques<sup>23</sup> for two pillow margin–interior pairs previously studied by H.S.S. and A.J.E.<sup>20</sup>. These REE data are presented in Table 1 and plotted relative to average C1 chondrite abundances<sup>23</sup> in Fig. 1. The small differences that exist within each pillow can be readily attributable to differences in phenocryst content and provide little evidence, in this particular instance at least, for differential movement of REE during low-grade alteration. Note that these particular pillow lavas show variations in Rb/Sr between margin and interior of between 20% and 400%<sup>21</sup>. The successful application<sup>24–27</sup> of the Sm–Nd system to the dating of Archaean igneous rocks of diverse metamorphic grade in general militate against metamorphic disturbance of Sm–Nd systematics.

## Sm/Nd ratios

The samples used in this study are from the type areas of the Theespruit (LUU), Komati (LUU) and Hoogenoeg (MFU) Formations, and were selected for optimum spread in Sm/Nd ratio coupled with preservation of original textures and relict

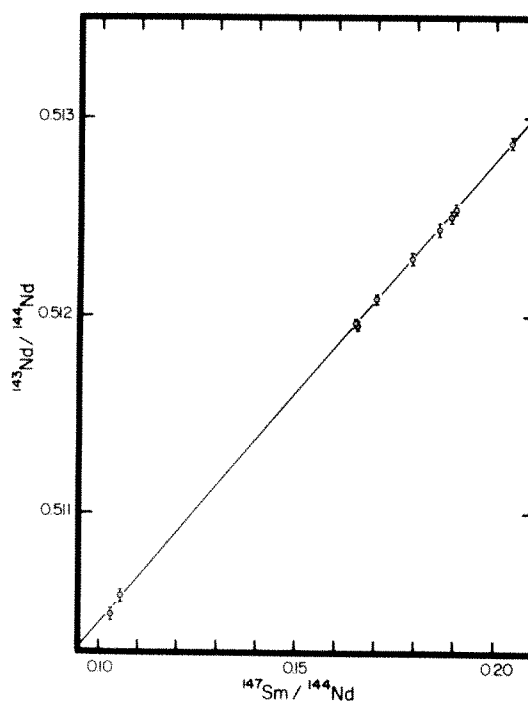


Fig. 2 Sm–Nd evolution diagram for the samples from the lower ultramafic unit. The data yield an age of  $3,540 \pm 30$  Myr and an initial  $^{143}\text{Nd}/^{144}\text{Nd}$  ratio of  $0.50809 \pm 4$  with errors quoted at the  $2\sigma_m$  level.

mineral content. None of the samples is totally fresh and typically, olivine is serpentinised, plagioclase sericitised and glass devitrified. Other common secondary minerals include calcite, tremolite, epidote, chlorite and talc. Sm and Nd concentrations and  $^{143}\text{Nd}/^{144}\text{Nd}$  ratios were determined (see refs 24 and 32 for analytical details) for 10 samples from the LUU and two from the MFU (Table 2). Sm–Nd data for samples from the LUU, which range from acid pyroclastics to spinifex-textured ultramafics, are plotted on a Sm–Nd evolution diagram in Fig. 2. The best-fit regression line<sup>28</sup> through the data points corresponds to an age of  $3,540 \pm 30$  Myr and an initial  $^{143}\text{Nd}/^{144}\text{Nd}$  ratio of  $0.50809 \pm 4$ . ( $^{147}\text{Sm}\lambda = 6.54 \times 10^{-12} \text{ yr}^{-1}$ ). The data for the seven mafic and ultramafic samples alone yield an age of  $3,510 \pm 60$  Myr and an initial  $^{143}\text{Nd}/^{144}\text{Nd}$  ratio of  $0.50813 \pm 7$  which are not significantly different from those obtained from all the LUU data.

**Table 2** Sm–Nd data for Onverwacht Group Volcanics

Sample	Rock type	Formation	Sm (p.p.m.)	Nd (p.p.m.)	$^{147}\text{Sm}/^{144}\text{Nd}^*$	$^{143}\text{Nd}/^{144}\text{Nd} \pm 2\sigma_m$
Lower ultramafic unit						
HSS-74	Sodic porphyry	Komati	2.129	12.42	0.1030	0.510487 $\pm$ 36
HSS-161	Acid tuff	Theespruit	2.033	11.59	0.1054	0.510570 $\pm$ 32
HSS-52B	Felsic pillow lava	Komati	4.891	17.79	0.1653	0.511950 $\pm$ 22
HSS-56	Basaltic lava	Komati	1.514	4.464	0.2040	0.512875 $\pm$ 32
R-14	Basaltic komatiite	Komati	1.115	3.551	0.1888	0.512504 $\pm$ 34
HSS-32	Basaltic komatiite	Komati	3.390	13.06	0.1649	0.511957 $\pm$ 22
HSS-88A	Peridotitic komatiite	Komati	1.267	4.251	0.1792	0.512292 $\pm$ 34
HSS-92	Peridotitic komatiite	Komati	0.5750	1.861	0.1858	0.512439 $\pm$ 34
HSS-95	Peridotitic komatiite	Komati	0.5490	1.736	0.1902	0.512541 $\pm$ 28
HSS-523	Peridotitic komatiite	Komati	1.318	4.659	0.1701	0.512084 $\pm$ 20
Mafic to felsic unit						
HSS-224	Tholeiitic basalt	Hoogenoeg	4.316	14.04	0.1849	0.512478 $\pm$ 22
19-J	Acid pyroclastic	Hoogenoeg	2.154	11.51	0.1125	0.510717 $\pm$ 24

\*  $^{147}\text{Sm}/^{144}\text{Nd}$  ratios are determined to a precision of 0.2% at the  $2\sigma_m$  level.

Inclusion of the data for the two samples from the MFU, though not plotted in Fig. 2, results in a less precise but indistinguishable age of  $3,570 \pm 50$  Myr and an initial  $^{143}\text{Nd}/^{144}\text{Nd}$  of  $0.50807 \pm 6$ . The Sm–Nd data satisfy the normal criteria for designating the regression line an isochron. These results do not resolve the time interval, represented by the Middle Marker Horizon, between the volcanic activity of the LUU and the MFU<sup>1</sup>.

If a single-stage mantle source history is assumed for the LUU volcanics from 4,550 Myr until 3,540 Myr ago and the  $^{143}\text{Nd}/^{144}\text{Nd}$  ratio 4,550 Myr ago was equal to that of the Angra dos Reis achondrite ( $0.50682$ )<sup>29</sup> then the time-integrated Sm/Nd ratio for this interval was  $0.297 \pm 0.005$ . This value is within error of those estimated from whole-rock isochron studies of the Isua<sup>25</sup>, Rhodesian<sup>24</sup> and Monroe Township<sup>27</sup> greenstone belt volcanics (Table 3) and together support the conclusion of DePaolo and Wasserburg<sup>30,31</sup> that Archaean mantle evolved with approximately chondritic Sm/Nd.

The peridotitic komatiites represent large degrees of partial melting whose primary abundance ratios of large ion lithophile elements may closely approximate those of their source region. Four samples of this rock type analysed in this study have Sm/Nd ratios which range from 0.270 to 0.302 and are within about 10% of the time-integrated Sm/Nd ratio of their source.

## Conclusions

Reliable initial  $^{87}\text{Sr}/^{86}\text{Sr}$  data for the Onverwacht volcanics could not be obtained from any of the samples available<sup>21</sup>. However, if the precise initial  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio determined by Jahn and Shih<sup>11</sup> for a LUU basaltic komatiite is assumed to be that characterising the LUU source region 3,540 Myr ago then an estimate of the time-integrated Rb/Sr ratio of the source can be obtained in a manner analogous to that used for the Sm/Nd ratio. The calculated time-integrated Rb/Sr ratio is  $0.034 \pm$

0.001, which is similar to that of 0.03 suggested previously<sup>31,32</sup> on the basis of the correlation of Nd and Sr isotope compositions in oceanic basalts.

Resolution of the temporal relationships between the Onverwacht Group and the Ancient Gneiss Complex must await further geochronological data for the latter which are of comparable precision to those reported here for the Onverwacht. Although the Onverwacht volcanics are of similar age to the Amitsoq gneisses<sup>33–35</sup> of West Greenland they do not represent the oldest known basic crust. The Isua metavolcanics which have been precisely dated by both U–Pb (ref. 35) and Sm–Nd methods<sup>25</sup> are some 200 Myr older.

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**Table 3** Sm–Nd Ages, initial  $^{143}\text{Nd}/^{144}\text{Nd}$  ratios and inferred time-integrated (ti) Sm/Nd ratios

Rock suite	Age (Myr)*	Initial $^{143}\text{Nd}/^{144}\text{Nd}^*$	(Sm/Nd) <sub>ti</sub> *
Isua <sup>25</sup>	$3,770 \pm 40$	$0.507831 \pm 46$	$0.306 \pm 0.006$
Onverwacht	$3,540 \pm 30$	$0.50809 \pm 4$	$0.297 \pm 0.005$
Lewisian <sup>26</sup>	$2,920 \pm 50$	$0.508959 \pm 49$	$0.311 \pm 0.003$
Rhodesia <sup>24</sup>	$2,640 \pm 140$	$0.50919 \pm 18$	$0.294 \pm 0.010$
Monroe Township <sup>27</sup>	$2,655 \pm 43$	$0.50924 \pm 6$	$0.304 \pm 0.003$

\* Errors quoted at  $2\sigma_m$ .

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# Hydrogen production from coal, water and electrons

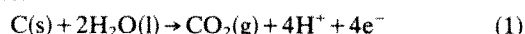
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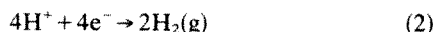
*Coals and other forms of solid carbonaceous fossil fuel are oxidised to oxides of carbon at the anode of an electrochemical cell and hydrogen is produced at the cathode, these gases being produced in relatively pure states. The reaction proceeds at very mild temperatures and at operating electrical potentials significantly lower than the thermodynamic potential of water electrolysis. Although the reaction is readily observable at room temperature, the observed activation energies and the expected decomposition temperatures of the presumed intermediates suggest that much more rapid and steadier oxidation rates might be achieved at higher temperatures in the range 200–600 °C.*

FOSSIL fuels and water are the major raw materials used to manufacture hydrogen although electrical energy produced by nuclear fission may become important for future production of hydrogen by electrolysis. Hydrogen is a key chemical for purifying petroleum of environmentally polluting ingredients; it is also important for converting the solid fossil fuel coal into clean fluid fuels. Mills<sup>1</sup> has specified hydrogen as the most costly ingredient in such conversions, the chemistry and technology of which are reviewed elsewhere<sup>1,2</sup>.

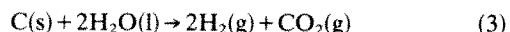
We report here a newly developed electrochemical process which converts coal and water into two separate gaseous products: one comprising essentially gaseous oxides of carbon and the other essentially pure hydrogen. The process chemistry takes place at mild temperatures (even room temperature) and the gaseous products are essentially free of impurities such as ash, tar and sulphur compounds. This new electrochemical gasification process involves the anodic oxidation of coal at an electrode for which we postulate the half-cell reaction of the carbon in coal:



in combination with a corresponding half-cell reaction at the cathode:



The net sum of these half-cell reactions (1) and (2) is the equation for the predominant reaction in the electrochemical gasification of coal:



This electrochemical gasification process, instead of producing a complex mixture containing H<sub>2</sub>, CO, CO<sub>2</sub> and impurities (as in conventional steam-carbon gasification), produces relatively pure streams of carbon oxides in the anode compartment and hydrogen at the cathode.

In principle, conventional water electrolysis requires a theoretical thermodynamic electrical energy input [ $\Delta F^\circ = -nFE^\circ$ ] of 56.7 kcal per mol of hydrogen and a corresponding theoretical driving potential of about 1.23 V whereas electrochemical gasification (equations (1)–(3)) requires according to thermodynamic principles only about 9.5 kcal of electrical energy and a reversible potential of only 0.21 V to produce 1 mol of hydrogen. The greatly lowered electrical energy requirement for the latter process results, of course, from the consumption of coal which can be viewed as supplying the additional electrons required by the process.

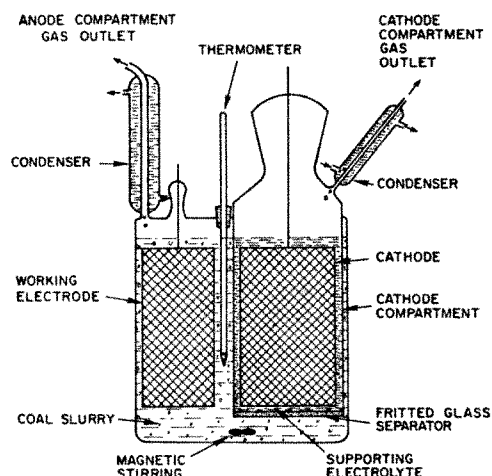
Of course, the stoichiometric reactions written above mask reaction mechanism(s) which as yet can only be speculative. Perhaps the primary electrode process is the discharge of OH<sup>−</sup> at the anode to produce a radical which attacks the coal. Overall, the carbon of the coal might also be viewed as an oxygen acceptor.

Conventional production of hydrogen from coal uses steam-carbon reactions to produce synthesis gas (CO and H<sub>2</sub>) by a strongly endothermic reaction which must be conducted at temperatures sufficiently high (~800 °C) to assure favourable equilibrium. Synthesis gas must then be purified to remove sulphur compounds and other impurities followed by reaction with water to shift the CO/H<sub>2</sub> ratio as required. Coal gasification technology is discussed in detail elsewhere<sup>2,3–5</sup>.

We have conducted electrochemical gasification of three coals, one lignite and one char (obtained from the Pittsburgh Energy Research Center of the US Department of Energy) by anodic oxidation in the apparatus of Fig. 1. Typical results recorded in Table 1 show substantial oxidation currents at applied potentials significantly lower than those necessary to electrolyse aqueous electrolytes, compared with control experiments made in the absence of coal. Hydrogen was produced at the cathode and carbon dioxide containing 3–7% carbon monoxide at the anode. Oxidation of the coal apparently requires contact with the anode—only background current is measured if the anode is surrounded by a porous membrane which prevents the close approach of the coal particles but permits transport of species dissolved in the anolyte.

In these early experiments, Montana Rosebud char gave higher current and seemed easier to oxidise anodically than the parent coal. Based on other experiments with active carbon, diamond and graphite we attribute this behaviour to the higher surface area, smaller particle size and more graphitic nature of the char as compared with the parent coal. Susceptibility of a carbonaceous particle to anodic oxidation may be related to the electrical conductivity of the particle.

Figure 2 shows the inter-relationship of oxidation current, temperature and coal-to-electrolyte concentration; increasing such concentrations or temperature causes current to increase as



**Fig. 1** Stirred slurry gasification cell. Supporting electrolyte H<sub>2</sub>SO<sub>4</sub>, 475 ml volume. Anode and cathode were Pt gauge 52 mesh with respective areas of 96.5 cm<sup>2</sup> and 158 cm<sup>2</sup>.



**Table 1** Comparison of oxidation rates of different coal samples

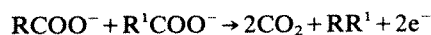
Coal	Potential of oxidation (V)	Coal concentration ( $\text{g cm}^{-3}$ )	Oxidation rate (mA)
Montana Rosebud char	0.78	0.475	8.20
North Dakota lignite	0.83	0.475	9.00
Pittsburgh coal	0.875	0.475	5.30
Illinois no. 6	0.875	0.475	3.00
Montana Rosebud coal	0.78	0.475	1.50

Electrolyte, 3.7 M  $\text{H}_2\text{SO}_4$ ; temperature, 23 °C; electrode area, 6.5  $\text{cm}^2$ ; coal slurry concentration, 0.475  $\text{g cm}^{-3}$ ; particle size, 250  $\mu\text{m}$  and below; experimental apparatus as in Fig. 1. Control experiments with no coal, lignite or char produced currents of only about 0.05 mA.

expected. The temperature dependence of oxidation rate leads to an estimate of apparent activation energy of about 10–12  $\text{kcal mol}^{-1}$  for North Dakota lignite at 1 V. Such low activation energy suggests that the rate determining step may not be a typical chemical reaction. Particle size effects have also been observed but they are complex and seem to be time dependent. The ash content, porosity and other properties of the particles may be size dependent and particle size is also related to momentum available for a particle to penetrate the nernstian layer at the anode. We have also obtained comparable results with electrodes other than Pt (such as graphite) and other electrolytes (such as HCl). The higher the potential the greater the oxidation current. As the coal is consumed by oxidation at a given potential the current diminishes as shown in Fig. 3 where current is plotted against the potential for various extents of coal consumption as a parameter. Anodic oxidation of carbon anodes has been investigated previously<sup>6</sup>. The controlled oxidation of purer carbons, whether by electrochemical or chemical means, results in the formation of surface oxides such as hydroxyl, carbonyl or carboxyl groups<sup>6–8</sup>. We believe that such oxides also form during the anodic oxidation of coal and, as they increase in concentration on the surface of the coal particles, the potential required for additional oxidation increases. These surface oxides of carbon decompose to gaseous oxides of carbon on heating and this is consistent with the experimental observation that as temperature increases lower potentials are required to produce the same rate of oxidation. The gradual decrease of the reaction rate may be attributed to a corresponding accumulation of chemisorbed oxygen in the form of surface functional groups on the coal<sup>6–8</sup>. Based on the known decomposition temperatures<sup>9</sup> for such surface compounds it should be possible to maintain a higher and steady oxidation rate at 200–600 °C, perhaps even at applied potentials lower than those reported here. Of course, such higher temperatures would entail operation at pressures above atmospheric.

Some experiments were performed in which, after significant consumption of the coal had taken place by anodic oxidation at about 100 °C, and the anodic current at 1 V had decreased to low values, the coal was removed from the electrolyte and heated to about 200 °C to decompose surface oxides. When this coal was returned to the anolyte, high oxidation currents were measured at 1 V again, and these currents were almost equal to those provided by virgin coal at the same conditions. Reactivity could also be restored by extracting reacted coal with hydrocarbon solvents.

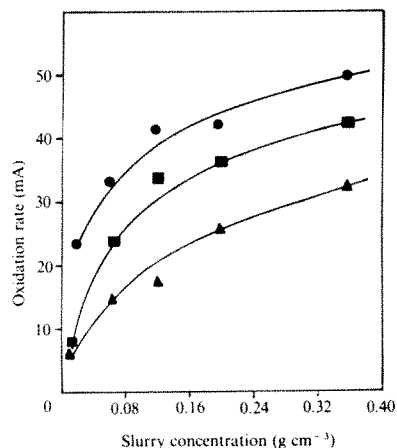
Surface carboxyl groups on coal or smaller carboxylated fragments can probably react through a Kolbe-type pathway to form aliphatic type hydrocarbons:



Surface free radicals,  $\text{R}\cdot$ , might also form by



Such reactions may be responsible for the tar-like substance that can be extracted from anodically oxidised coals using hydrocarbon solvents. For example, acetone extracted about 5% of the weight of 25%-consumed NDL to form a dark, reddish-

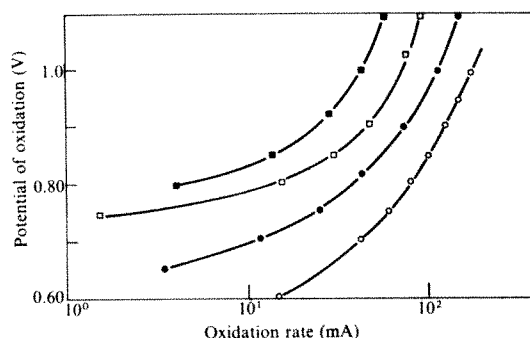


**Fig. 2** Effect of coal concentration on the oxidation rate. (North Dakota lignite; particle size, 44  $\mu\text{m}$  and below; supporting electrolyte concentration, 4.13 M  $\text{H}_2\text{SO}_4$ ; oxidation potential, 1.00 V; experimental apparatus as in Fig. 1 except anode area 6.5  $\text{cm}^2$ . ●, 78 °C; ■, 59 °C; ▲, 39 °C.

brown solution, whereas a similar treatment of the parent coal produced an extract that remained almost clear.

### Gases produced and the current efficiency

During oxidation of ND lignite at 114 °C and at potentials near 0.85–1.0 V the gas produced at the cathode was essentially pure  $\text{H}_2$ . Based on 12 experiments in each of which about 100  $\text{cm}^3$  of  $\text{H}_2$  was collected, the mean current efficiency (defined as: mol  $\text{H}_2$  collected/mol  $\text{H}_2$  corresponding to coulombs passed) was 1.00 with a standard deviation of  $\pm 0.022$ . The gas produced within the anode compartment was almost pure  $\text{CO}_2$  with small amounts of CO ranging from about 7% early in the electrolysis (about 1,870 C passed, equivalent to about 0.34% of coal consumed) to a steady-state value of about 3% attained at about 3,740 C passed (equivalent to about 0.68% of coal consumed). The volume ratio of the gases collected at the cathode to those at the anode ranged from  $\sim 3.5$  to  $\sim 8$ ; the higher ratios were obtained at the beginning of the experiment but then decreased. According to reaction (3) the gas ratio should be about 2. In the present conditions, however, a significant portion of the carbon oxides presumably remained bound to the coal (probably as  $-\text{COOH}$ ,  $-\text{CHO}$  and  $-\text{CH}_2\text{OH}$  groups) and this would account for the gas volume ratios  $> 2$ . The decrease in these ratios as an experiment progresses may be attributed to the build-up of oxygen on the carbon surface until a steady-state saturation is reached. Higher temperatures should cause more oxides of carbon to be liberated from the coal with an attendant lowering of the ratio of cathode-to-anode gas production rate. Some electrolyte-soluble, (probably oxygenated) organic compounds



**Fig. 3** Effect of potential on the oxidation rate as the reaction proceeds. (North Dakota lignite; coal slurry conc., 0.069  $\text{g cm}^{-3}$ ; supporting electrolyte, 5.60 M  $\text{H}_2\text{SO}_4$ ; particle size, 125–149  $\mu\text{m}$ ; temperature, 114 °C experimental apparatus, as in Fig. 1 with anode area 96.5  $\text{cm}^2$ . Total carbon consumed: ○, 0.156%; ●, 6.35%; □, 21.1%; ■, 29.2%.

were also produced as indicated by total organic carbon assays of the filtered electrolyte after extended anodic oxidation of coal.

A qualitative but sensitive mass spectrometric analysis was made of the gases produced at both anode and cathode. Note that no lines were observed for molecular weights corresponding to  $\text{SO}_2$  or  $\text{H}_2\text{S}$ , even though the parent lignite contains significant sulphur.

We can compare the efficiency of our process (coal-consuming water electrolysis) with ordinary water electrolysis. In water electrolysis the energy required to split the water molecule is supplied solely by electricity, whereas in our new process the required energy is supplied only in part by electricity with the balance arising by way of the concomitant anodic oxidation of coal. The following quantitative development gives a first order approximation of how much energy comes from each such source and thereby provides a rough feeling for efficiency. The energy consumed by conventional water electrolysis conducted at a potential of  $E_2$  to produce  $N_{\text{H}_2}$  moles of  $\text{H}_2$  is  $2N_{\text{H}_2}FE_2$  whereas the energy required by the present process operating at a potential of  $E$  is:

$$E \int_0^t i \, dt + N_{\text{C}}(-\Delta H)$$

where  $E$  is the potential applied across the cell;  $i$  is the current; and  $t$  is time  $N_{\text{C}}$  is the number of mol of carbon consumed and  $\Delta H$  is the enthalpy of combustion of carbon to  $\text{CO}_2$ .

The foregoing expression can be simplified by assuming a constant operating potential  $E$ , and noting that:

$$N_{\text{H}_2} = \int_0^t i \, dt / 2F \quad \text{and} \quad N_{\text{C}} = 1/2 N_{\text{H}_2}$$

where  $F$ , the Faraday constant, is  $96,500 \text{ C equiv.}^{-1}$ .

Eliminating  $\int_0^t i \, dt$  and  $N_{\text{C}}$  the expression for total energy consumption by our process becomes

$$2FN_{\text{H}_2}E + 1/2N_{\text{H}_2}(-\Delta H)$$

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The relative energy usage (REU) is accordingly:

$$\begin{aligned} \text{REU} &= \left( \frac{\text{ordinary electrolysis}}{\text{coal-assisted electrolysis}} \right) \\ &= \frac{2N_{\text{H}_2}FE_2}{2FN_{\text{H}_2}E + 1/2N_{\text{H}_2}|\Delta H|} \\ &= E_2/(E + |\Delta H|/4F) \end{aligned}$$

Inserting  $|\Delta H| = 94,100 \times 4.18 \text{ J mol}^{-1}$  and the value of  $F$  gives:

$$\text{REU} = E_2/(E + 1.02)$$

Practical values of  $E_2$  for conventional electrolysis are about 2 V whereas values of  $E$  observed in the present work have ranged from  $\sim 0.8$  to  $\sim 1.0$  V at room temperature. This means that the total energy consumption is about the same ( $\text{REU} \approx 1$ ), per unit of hydrogen produced, for ordinary water electrolysis and for coal-assisted water electrolysis conducted in the experiments near room temperature reported here. In the case of electrochemical gasification to hydrogen, however, about half the required energy comes directly from coal and half from electricity. We expect that the total energy requirement for coal-assisted water electrolysis can be lowered further by carrying it out at higher temperatures thereby permitting operation at lower potentials ( $E$ ) than 0.8–1.0 V. Note that we have treated coal as pure carbon and assumed it is completely oxidised to  $\text{CO}_2$  at the anode in the above approximate assessment of REU. Such simplification ignores the complications arising from the variable hydrogen and oxygen content of different coals and the question of the different relative proportions of  $\text{CO}$ ,  $\text{CO}_2$  and small amounts of non-gaseous carbon oxides that may be formed at different operating conditions.

The results presented here represent a new technical concept for preparation of hydrogen from the electrochemical reaction of water and coal. Additional results will be reported elsewhere.

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# letters

## The origin of baryons in the Universe

THERE are several clues to the history of the early Universe. The 3-K microwave background indicates that the Universe was highly isotropic when it was about a half million years old, and the abundance of  $^4\text{He}$  (also  $\text{D}$ ,  $^3\text{He}$  and  $^7\text{Li}$ ) indicates that nucleosynthesis was taking place when the Universe was about three minutes old. These two facts are strong evidence that the Universe began from a hot big bang<sup>1</sup>. In addition, Hawking, Ellis and Penrose have proved (within general relativity) that the existence of the 3-K radiation implies the Universe must have been singular in its past<sup>2,3</sup>. The existence and clustering of galaxies indicate there were some deviations from homogeneity in the early Universe. Perhaps the most curious fact about the

Universe is that it is composed almost entirely of matter (the Universe contains negligible amounts of antimatter) and that the number of baryons per photon ( $\sim$  baryon/entropy ratio) is between  $10^{-10}$  and  $10^{-8}$  (ref. 4). If baryon number is absolutely conserved, then it follows that the baryon number of the Universe must be viewed merely as an initial condition. Recent ideas in particle physics when applied to the early Universe may explain how an initially baryon-symmetrical (zero net baryon number) Universe could have evolved into one with net baryon number. Here, we will discuss these ideas and other interesting astrophysical implications.

The striking success of the Weinberg-Salam  $\text{SU}(2) \times \text{U}(1)$  gauge theory of the weak and electromagnetic interactions<sup>5</sup> and of the  $\text{SU}(3)$  colour gauge theory of the strong interactions<sup>6,7</sup> has

motivated grand unified gauge theories of the strong, weak and electromagnetic interactions<sup>8-12</sup>. A common feature of all these grand unified theories is baryon non-conservation mediated by a very heavy (Higgs and/or gauge) boson(s) of mass  $\sim 10^{10}$ – $10^{16}$  GeV  $c^{-2}$ . In addition, these theories naturally violate CP (particle-anti-particle symmetry).

At present energies (even those of the largest proposed particle accelerators), these baryon non-conserving processes are not important; however, in the early Universe, when  $kT \geq m_x c^2$  ( $m_x$  is the mass of the boson which mediates baryon non-conserving reactions) these processes would have been roughly the same strength as the other interactions and an initially baryon-symmetrical Universe could have evolved into one with net baryon number. Several authors<sup>13-16</sup> have pointed out that in addition to CP and B violations one must also have departures from thermal equilibrium to produce baryon asymmetry. Departures from equilibrium occur naturally in the early Universe when reaction rates cannot keep pace with the expansion rate of the Universe. Several schemes have been proposed<sup>13,14,16-19</sup> which produce a baryon/photon ratio of  $\sim 10^{-10}$ – $10^{-8}$ . We shall describe the one proposed by Weinberg<sup>16</sup>, and discuss the astrophysical consequences of cosmological baryon production and a new hybrid scheme using an earlier idea of Hawking<sup>20</sup> as well as the new unification theories.

In the Weinberg scheme, the Universe begins as a baryon-symmetrical hot soup containing all the fundamental particles (leptons, quarks, photons, X-bosons). When the lifetime of the X-boson is approximately the age of the Universe,  $kT$  is less than  $m_x c^2$ , so that the X- and  $\bar{X}$ -bosons freely decay (their decay products are not energetic enough to regenerate them). At this temperature baryon non-conserving reactions have become ineffective (reaction rates < expansion rate) so that any baryon number generated will not be destroyed. The decay products of the  $\bar{X}$ -bosons have a mean net baryon number  $B_{\bar{X}} \neq -B_X$ ,  $B_X$  = the mean net baryon number of the decay products of X-bosons (this is permitted by CP and B violations). Therefore, the Universe acquires a baryon/photon ratio proportional to  $(B_X + B_{\bar{X}})$ , which is itself proportional to the size of the CP violation. From this point forward the Universe evolves with the baryon/photon ratio remaining constant or decreasing with the generation of additional entropy or with the decay of baryons.

Because grand unified theories do not conserve B, they also predict that the proton is unstable. In the SU(5) and O(10) unification theories,  $\tau_p \sim \alpha^{-2} m_x^4 / m_p^5 \sim 10^{32 \pm 1}$  yr ( $\alpha$  ~ fine structure constant  $\cong 1/137$ )<sup>21,22</sup>. The current lower limit on the proton lifetime is  $10^{29}$  yr and comes from the Case-Wit-Irvine neutrino experiment in South Africa<sup>23</sup>. Lande *et al.* are using the neutrino telescope at Homestake Mine in Lead, South Dakota (for this experiment neutrinos are the background!) to explore lifetimes of  $\sim 10^{31}$  yr (by mid-1979) and hope to be able to have developed a sensitivity to lifetimes of  $\sim 10^{32}$  yr within 2 yr (K. Lande, personal communication). A positive result from a proton decay experiment would be strong evidence for baryon generation in the early Universe. In addition, if the Universe is open, such a result means that it will eventually be devoid of baryonic material.

In the gravitational instability theory of galaxy formation and clustering, small initial density fluctuations grow with time and eventually become of the order of unity, leading to the formation of galaxies and clusters of galaxies<sup>24</sup>. The initial fluctuations are of two types: adiabatic and isothermal. In an adiabatic fluctuation both the matter and radiation are perturbed and are related by  $(\delta\rho/\rho)_{\text{matter}} = 3(\delta T/T)_{\text{radiation}}$ . In an adiabatic fluctuation the baryon/entropy ratio remains constant. In an isothermal fluctuation the radiation does not participate in the perturbation ( $\delta T/T = 0$ ) and so the baryon/entropy ratio is not constant. The adiabatic perturbations are subject to damping (while the matter and radiation are coupled) and only fluctuations on mass scales  $\geq 10^{12} M_\odot$  will survive and grow to the order of unity. Isothermal perturbations are not subject to damping, and fluctuations on mass scales  $\geq 10^5 M_\odot$  will grow to the order of unity. If the initial fluctuations were adiabatic then the 3-K background should

show variations  $\Delta T/T \sim 10^{-3}$  on angular scales of  $\sim 30$  arc s. Such variations have not been seen and are just below the sensitivity level of current experiments<sup>25</sup>.

If the baryons in the Universe were generated as outlined above, the baryon/entropy ratio would depend only on microphysics (the grand unified theory). Therefore, the initial temperature fluctuations in the Universe would not give rise to baryon/entropy fluctuations (unless the fluctuations were so large that some parts of the Universe were never hotter than  $m_x c^2/k$  and no net baryon number was generated). Any initial density perturbations must be adiabatic rather than isothermal. This fact is related to the problem of galaxy formation and clustering in the Universe.

One way around the elimination of initial isothermal fluctuations is through primordial black holes. Hawking has shown that a black hole of mass  $m$  (in grammes) should emit a spectrum of particles like a black body with a temperature of  $\sim 10^{26}$  K  $m^{-1}$  (ref. 26). As a black hole radiates, it loses mass and ultimately evaporates in a time  $\sim 10^{-26} m^3 N^{-1}$  s ( $N$  ~ number of particle species it can emit)<sup>27</sup>. As it is evaporating its temperature increases; when  $kT \sim m_x c^2$  a black hole can emit X- and  $\bar{X}$ -bosons (in equal numbers). When these bosons decay they will produce a net baryon number  $\sim (B_X + B_{\bar{X}}) \times (\text{number of X, } \bar{X}\text{-pairs emitted})$ . All black holes with an initial mass  $m$  such that the corresponding temperature is  $\leq m_x c^2/k$  ( $m \geq 10^{13} m_x^{-1}$ ,  $m_x$  in GeV) will eventually produce the same number of X,  $\bar{X}$ -pairs and hence the same net baryon number. (This is because no X,  $\bar{X}$ -pairs will be produced until the mass of the black hole has decreased such that its temperature  $\sim m_x c^2/k$ .) The rest of the mass of an evaporating black hole will eventually be converted into photons (entropy), so that the baryon/entropy ratio created by an evaporating black hole is proportional to  $m^{-1}$ . The entropy and baryon number of the Universe could be produced from an appropriate primordial spectrum of black holes (Hawking first suggested baryon and entropy production by primordial black holes, although he did not elaborate on a mechanism<sup>20</sup>). In addition, as the baryon/entropy ratio produced by an evaporating black hole depends on its mass, the distribution and mass spectrum of primordial black holes could lead to both isothermal and adiabatic initial density fluctuations in the Universe. For example, suppose the Universe was initially isothermal and without net baryon number. Let the net baryon number be produced by a non-uniform spatial distribution of primordial black holes of mass  $m$ . If the entropy added by the evaporation of the black holes is small (let  $m$  be  $\sim 10^{13} m_x^{-1}$  g) the density fluctuations caused by the spatial distribution of black holes will be nearly isothermal. For some values of  $m_x$ , baryon production by other routes is negligible because when the X and  $\bar{X}$  decay baryon non-conserving collisions are still effective and any baryon excess produced is neutralised. In these cases primordial black holes can act as 'seeds', evaporating after nm-conserving collisions become ineffective and thereby generating a baryon excess that is not neutralised.

In conclusion, we can say that recent developments in the unification of elementary particle interactions by gauge theories may provide an explanation for what seemed to be an arbitrary initial condition—the baryon/entropy ratio. In addition, if this explanation is correct, it is directly relevant to the question of initial density fluctuations and galaxy formation.

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## IUE observations of the symbiotic star CH Cygni during an active phase

THE spectrum of CH Cygni usually has the appearance of a normal M6 III star, but at intervals of several years, it has outbursts during which it shows the characteristics of a symbiotic star, that is a blue continuum extending shortward of the Balmer discontinuity, and emission lines of He I, Fe II, [Fe II], [S II], [O III]; the Balmer lines and the H and K lines of Ca II present emission wings. The observations of CH Cygni reported here were made to determine whether a symbiotic star is a binary system composed of an M6 giant and a hot subdwarf, or whether it is a cool star surrounded by a thick corona.

Outbursts were observed: in September 1963 (ref. 1), the star had already returned to normal by August 1965; in June 1967 (ref. 2) the spectrum was followed by Faraggiana and Hack<sup>3</sup> until December 1970 when the star returned to normal; another

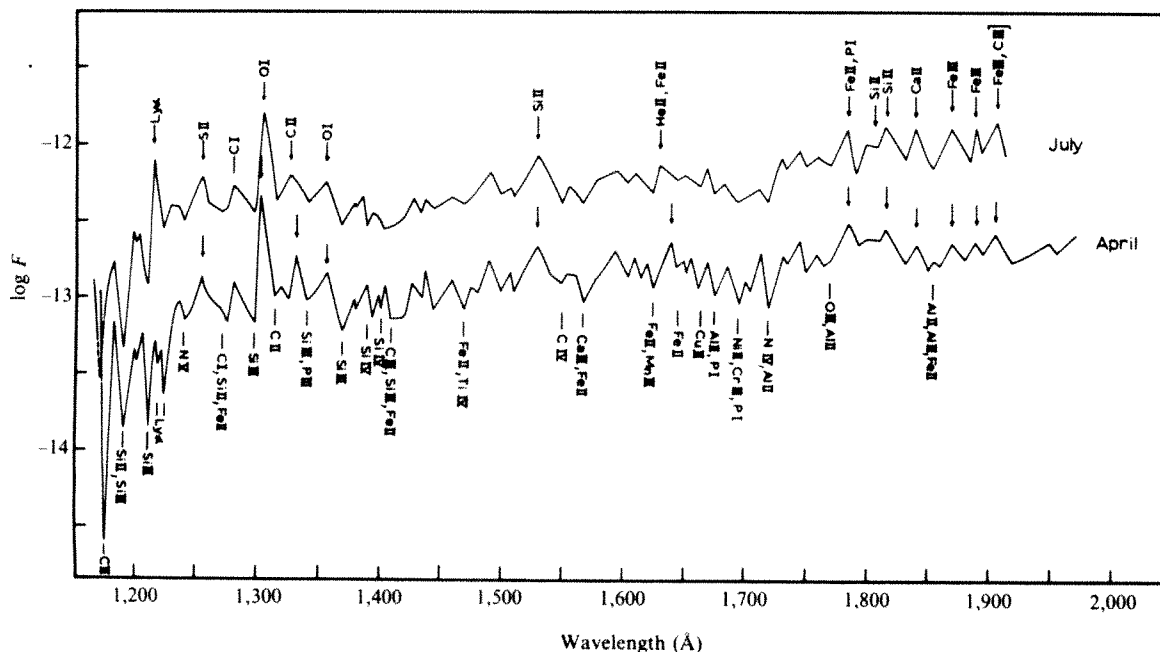
outburst was observed in August 1977 (refs 4, 5), the spectrum was observed by Faraggiana with the coude spectrograph at the 152-cm telescope of the Haute Provence Observatory, and in June and September 1978 still had the characteristics of a symbiotic star. Our observations of the UV spectrum of CH Cygni reported here, made with IUE on April and July 1978 were therefore taken during its active phase.

The most obvious hypothesis for explaining symbiotic stars is that they are binary systems formed of a late-type giant and a hot subdwarf which occasionally has outbursts. Or the cool star may be surrounded by an extended and thick corona heated by shock waves, which is supposed to be responsible for the blue continuum and the emission lines<sup>6,7</sup>. A third hypothesis<sup>8</sup>, that a hot star is surrounded by a cool, optically thick envelope, was discarded by the observations of CH Cygni in 1967-70 (ref. 3) because they gave clear evidence that the blue continuum was filling up the M6 photospheric lines, a distinctive phenomenon which was again observed in the spectra obtained by Faraggiana in June and September 1978 (personal communication).

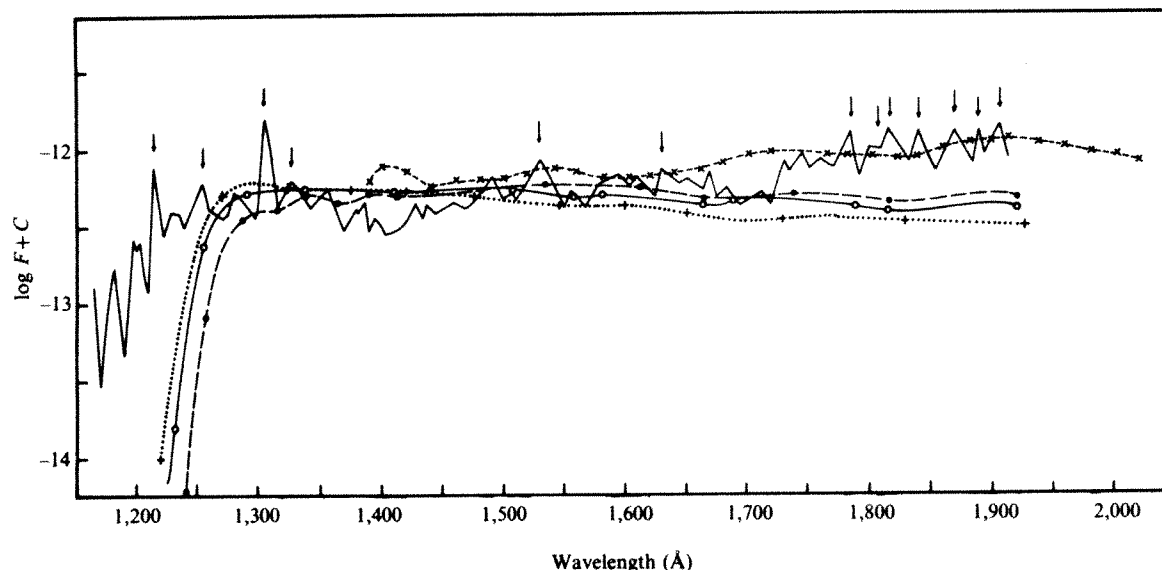
Several symbiotic stars present orbital motions; however, CH Cygni does not show any evidence of periodic radial velocity variations. Instead, our observations supported the hypothesis that shock waves heat the extended envelope surrounding the M6 giant star. Photometric observations by Slovak and Africano<sup>9</sup>, however, indicate that CH Cygni presents a rapid flickering, characteristic of the cataclysmic variables, most of which are close binary systems. According to Slovak and Africano<sup>9</sup>, the flickering provides the strongest evidence for the interpretation of CH Cygni as a binary system undergoing mass transfer.

To decide which hypothesis could explain the spectrum of CH Cygni, it was observed with IUE, in the low resolution mode and in the short-wave range on 22 April (using the 3" aperture) and on 31 July 1978 (using the large aperture, 10" × 20").

The UV spectrum of CH Cygni is characterised by an almost flat continuum from  $\lambda 1250$  to  $\lambda 1700$  with a mean flux of about  $5 \times 10^{-13} \text{ erg s}^{-1} \text{ cm}^{-2} \text{ \AA}^{-1}$  (determined from the July spectrum, observed with the large aperture), by a very strong emission at  $\lambda 1302$  (the flux at the centre of the emission is about 5 and 4 times the flux in the adjacent continuum, in April and July respectively) due to O I, and by several fainter emissions, which in many cases can be barely distinguished from high points on the continuum; moreover, several absorption lines are present. The energy distribution in the continuum at  $\lambda > 1700$  is strongly



**Fig. 1** The spectra of CH Cygni observed in April and July. The ordinates are  $\log F$ , where  $F$  is the flux at the Earth ( $\text{erg cm}^{-2} \text{ s}^{-1} \text{ \AA}^{-1}$ ) given by the relation  $F_{\lambda} = N_{\lambda} S_{\lambda}^{-1} t^{-1}$ , where  $N_{\lambda}$  is the IUE flux number,  $S_{\lambda}$  the camera sensitivity and  $t$  the exposure time in seconds. The emissions are indicated by arrows.



**Fig. 2** The far UV spectrum of CH Cygni, observed on 31 July 1978, is compared with that of Beta Lyrae (S2/68 observations) and with the Kurucz, Peytremann and Avrett blanketed models.  $\times$ — $\times$ , Beta Lyrae;  $\bullet$ — $\bullet$ , KPA model,  $T_e = 10,000$  K,  $\log g = 4.5$ ;  $\circ$ — $\circ$ ,  $T_e = 11,000$  K,  $\log g = 4.5$ ;  $+$ — $+$ ,  $T_e = 13,000$  K,  $\log g = 4.5$ . The arrows above the CH Cygni spectrum indicate the position of the emission lines.

affected by the presence of the emissions. Their blending produces in this region a peculiar energy distribution, very similar to that previously observed for Beta Lyrae<sup>10</sup> with experiment S2/68 (spectral resolution about 35 Å) aboard the European satellite TD-1.

The two spectra observed in April and July are almost identical, except for a constant difference in the flux, which is four times lower in April, a difference probably due exclusively to the light loss through the 3" aperture<sup>11</sup> (Fig. 1). Moreover, the July spectrum presents a strong emission at Ly $\alpha$ , which is of geocoronal origin, because it fills up the whole height of the slit, as shown by inspection of the photowrite. No appreciable variations are evident, either, in the region  $\lambda\lambda 3500$ – $5000$  Å, at least from a visual inspection of the spectra taken in June and September 1978.

As it is very difficult to trace a reliable continuum through the various absorption and emission features, we have considered an average curve passing through the majority of these features, but below the strongest emissions and above the strongest absorptions (Fig. 2). We then compared this with the spectra of several stars observed with S2/68 (ref. 12), and with several theoretical models<sup>13</sup>. The best fit along the whole region 1380–1900, common to IUE and S2/68 observations, is obtained with Beta Lyrae, whose spectrum is also characterised by the presence of several strong emissions. As Beta Lyrae has been observed in the high resolution mode with IUE, the identification of the emissions in its spectrum has been a useful guide to the identification of the emissions in the spectrum of CH Cygni. Comparison with normal unreddened stars indicates that the best agreement is found with B9–A0 stars, but only in the region  $\lambda < 1750$  Å, because of the distortion due to the emissions. Atmospheric models for  $T_e \approx 10,000$  K or 11,000 K agree with the observed energy distribution in the region  $\lambda\lambda 1275$ – $1750$  Å (Fig. 2). At  $\lambda > 1750$  Å, the continuum is distorted by the blending of several strong emission features, and at  $\lambda < 1,275$  Å the flux decrease is slower than that predicted by the models. This latter behaviour indicates that the Ly $\alpha$  absorption is much fainter in CH Cygni, an effect we attributed to the presence of geocoronal and stellar Ly $\alpha$  emission.

The magnitude difference  $\Delta m$  with the standard star HD168905,  $B2.5$  V,  $V = 5.24$ , observed with IUE with the large aperture (Selvelli, personal communication), ranges from +9.5 at  $\lambda 1170$  to +6.0 at  $\lambda 1900$ . The magnitude difference with Vega (observed with S2/68 (ref. 12) ranges from +10.3 at  $\lambda 1450$  to +9.3 at  $\lambda 1900$ .

The main contributors to the blends are given in Fig. 1. Absorption lines of multi-ionised atoms like  $\lambda 1175$  C III,  $\lambda 1550$  C IV,  $\lambda 1718$  N IV,  $\lambda 1240$  N V, probably  $\lambda 1767$  and  $\lambda 1771$  O III,  $\lambda 1400$  Si IV are present. All the emission lines, on the contrary, are due to transitions to the ground level of neutral or once-ionised atoms (like  $\lambda 1253$  S II, multiplet 1 (ref. 14);  $\lambda 1281$  C II, 5;  $\lambda 1303$  and  $\lambda 1355$  O I, 2;  $\lambda 1335$  C II, 1;  $\lambda 1533$  Si II, 2;  $\lambda 1785$  P I, 1;  $\lambda 1808$  Si II, 1;  $\lambda 1817$  Si II, 1) and to the strongest lines of iron twice-ionised. The emission at  $\lambda 1640$ , attributed to He II, probably receives an important contribution from the Fe II lines of multiplets 8 and 68, which have the second strongest intensities in this spectral region, after those of multiplet 191, which is a possible explanation for the emission at  $\lambda 1785$ , together with P I.

These observations do not enable us to decide between the two hypotheses outlined above. The UV continuum seems to be the continuation of the optical violet continuum observed during outbursts, whose colour temperatures was found to equal  $\sim 10,000$  K (ref. 3). This temperature is not sufficient to explain the presence of the absorption lines of multi-ionised atoms. If we assume that the UV continuum is due to an increase in brightness of the hypothetic companion or that it arises in a hot corona surrounding the cool star and which becomes optically thick during the active phases then we must assume that mechanical energy, liberated during the outburst, is responsible for the high degree of ionisation of the gas. In contrast the emission lines should form in an extended envelope. The presence of this envelope, where hydrogen is mostly ionised, is indicated by the strong emission of O I at  $\lambda 1302$ . In fact Ly $\beta$  and  $\lambda 1025.77$  O I have almost the same high excitation potential: therefore, Ly $\beta$  emission overpopulates the upper level of  $\lambda 1025$  O I; hence by cascade, emissions at  $\lambda 11287$ ,  $\lambda 8446$  and  $\lambda 1302$  are explained<sup>15</sup>.

It would be necessary to observe the UV spectrum of CH Cygni in periods of quiescence to understand better the nature of the companion, if present, and to study the conditions of the envelope far from the effects of the outbursts.

Note that CH Cygni has been observed again with IUE on 11 March 1979 in the low resolution mode. The spectrum is identical with that observed on July 1978, but the flux is increased by a factor 1.6 over the whole spectral range 1,200–1,900 Å.

This research is based on observations by the IUE collected at the Villafranca Satellite Tracking Station of the ESA. I thank all the staff of the Villafranca Station for their assistance in observ-

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## Temperature structure of the uranian upper atmosphere

FROM observations of the occultation of the star SAO158687 by Uranus on 10 March 1977<sup>1,2</sup>, we have determined the temperature structure of its upper atmosphere at two locations on the planet. These are the first temperature measurements within the  $10^{-4}$ – $10^{-2}$  mbar range, which lies several scale heights above the region probed by IR measurements. Although this occultation was noted for the unexpected discovery of the uranian rings<sup>3–5</sup> and ring occultations were observed by several groups<sup>6</sup>, an occultation by the planet itself was seen from only two observatories—the Kuiper Airborne Observatory (KAO) and Cape Town. Here we present the first results from the KAO data: the Cape Town results will be discussed elsewhere<sup>7</sup>.

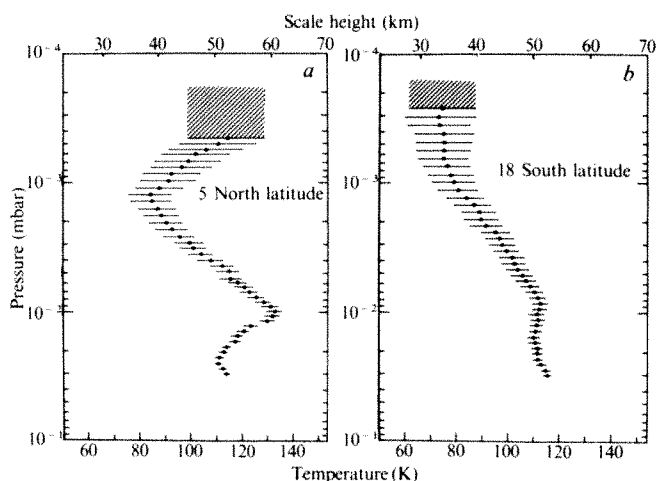
The data we obtained with the 91-cm telescope aboard the KAO consist of continuous records of the starlight intensity, recorded simultaneously at three wavelengths (6,190; 7,280 and 8,520 Å) with a time resolution of 10 ms. These wavelengths, which correspond to methane absorption bands in the spectrum of Uranus, were chosen to enhance the signal-to-noise ratio of the data by discriminating against the background light from Uranus<sup>2</sup>. The immersion and emersion records contained many 'spikes' as were observed during occultations by Jupiter and Neptune<sup>8</sup>. A variable component of scattered moonlight, amounting to a few per cent of the unocculted stellar intensity, was removed from the light curves before analysis. Details of our observations are given elsewhere<sup>3,9</sup>.

The light curves were analysed by the inversion method of French *et al.*<sup>10</sup> to yield the number density,  $n(h)$ , pressure,  $p(h)$ , and scale height,  $H(h)$ , of the uranian atmosphere as a function of height  $h$  in the atmosphere. [Note that inversion of occultation light requires the validity of certain general assumptions; see refs 11–16.] The temperature  $T(h)$  was determined through the relation  $T(h) = \mu g H(h)/R$ , where  $\mu$  is the mean molecular weight of the atmosphere,  $g$  is the local gravitational acceleration and  $R$  is the universal gas constant. Here we have set  $g = 830 \text{ cm s}^{-2}$ , which corresponds to a rotation period of 24 h, and  $\mu = 2.2 \text{ g mol}^{-1}$ , which corresponds to an atmosphere of hydrogen and helium in their solar abundances. At these altitudes the concentration of methane would be much too low to significantly affect the mean molecular weight<sup>17</sup>.

The temperature–pressure relationships obtained from the immersion and emersion data for our 7,280 Å channel are

shown in Fig. 1. The shaded region in the upper part of each figure represents the initial temperature and its uncertainty obtained from the initial conditions that were used to begin the numerical inversion of the data. The temperature profiles terminate at high pressures (lower altitudes) when the light flux has become small enough for the uncertainty in the zero flux level to produce a significant error in the profiles. The error bars on the profiles are due to the effects of noise in the original light curves. The scatter of adjacent points is less than the length of the error bars because the photon noise in the light curve produces correlated errors in the profiles, as discussed by French and Elliot<sup>18</sup>.

The temperature profiles in Fig. 1 show peak-to-peak variations of 45 K for immersion and 35 K for emersion. The temperature variations at higher altitudes are probably an artefact of the inversion procedure<sup>18</sup>, so that the actual temperature variations may be somewhat less than shown in Fig. 1 (particularly for emersion). Hence the peak-to-peak variations of



**Fig. 1** Temperature–pressure relationships for the upper atmosphere of Uranus. The temperature of the upper atmosphere of Uranus is plotted against pressure for *a*, the immersion data and *b*, the emersion data. The mean temperature of each profile is about 100 K, which shows that Uranus has a temperature inversion between  $10^{-3}$  mbar and the  $10^{-2}$  mbar level probed by IR measurements, where the temperature is 58.5 K. Both profiles show wave-like temperature variations, which may be due to dynamical or photochemical processes. Nearby points differ by less than the calculated error bars because the photon noise in the original data produces correlated errors in the temperature–pressure relationships. (Data from KAO Ch. 2).

35–45 K must be considered as maximum values. However, we believe that a large component of these variations is real and indicates that the atmosphere of Uranus is not isothermal in this region for three reasons: (1) the same variations occur in the temperature profiles derived from all three of our photometric channels; (2) the variations are much larger than the error bars, indicating that they cannot be attributed to photon noise in the data; and (3) similar variations occur in the profiles obtained from the Cape Town data. The variations may be due to dynamical processes in the atmosphere or photochemical layering, as discussed below.

The temperature gradients are sub-adiabatic everywhere, although the gradient near  $p = 10^{-3}$  mbar is very close to the dry adiabatic gradient. The condensation boundaries for  $\text{CH}_4$ ,  $\text{NH}_3$  and  $\text{H}_2\text{O}$  all lie well to the left of the profile, if we assume that the ratio of the number densities of these molecules to the hydrogen number density is fixed at the saturation value of the temperature minimum at the 100-mbar pressure level.

The mean temperatures of the region probed by the occultation events, obtained by averaging the points in each profile,



are 109 K for immersion and 96 K for emersion. We also obtained mean temperatures of 122 K and 110 K by fitting a model occultation curve<sup>19</sup> for an isothermal atmosphere to the data, but prefer the mean temperatures obtained from the inversion profiles since the atmosphere is definitely not isothermal in this region. The higher temperature obtained from the immersion data may be due to the 'phase' of the temperature variations and not represent an actual difference between the mean temperatures that would be obtained for these two regions by averaging over a sufficiently long time or a larger altitude range. We note that immersion occurred just before sunrise at latitude 4.5° north and emersion occurred just after sunrise at latitude 18.1° south. The subsolar latitude at the time of the occultation was 51.0° north, and the phase angle was 2.4°.

Summarising our conclusions about the thermal structure of Uranus in the  $10^{-4}$ – $10^{-2}$  mbar region: (1) the mean temperature is about 100 K, (2) the atmosphere is not isothermal and (3) the temperature variations are larger in the region sampled by immersion than that sampled by the emersion event.

The mean temperature of 100 K at the occultation level is about 40 K higher than the IR temperature, implying that a temperature inversion occurs between the  $10^2$  and  $10^{-3}$  mbar levels. The cause of the inversion may be heating of the upper atmosphere by methane, as postulated for the models of Wallace<sup>20</sup>, however, his model temperatures are warmer than the temperatures found here. His 'saturated' model is in better agreement than his 'supersaturated' model. Another possible explanation for the temperature inversion is the dissipation of energy by dynamical processes, if these are the cause of the observed temperature variations. However, the problem with this mechanism is that the possible dynamical processes identified so far would heat the atmosphere to a temperature well above the observed value<sup>21</sup>.

**Table 1** Temperatures of Uranus and Neptune

	Uranus	Neptune
Mean distance from Sun	19.2 AU	30.1 AU
Effective temperature (IR, $p \sim 10^2$ mbar)	$58.5 \pm 2$ K (ref. 22)	$59.7 \pm 4$ K (ref. 22)
Occultation temperature ( $p \sim 10^{-3}$ mbar)	100 K	140 K (refs 25–27)
Heating mechanism at $p \sim 10^{-3}$ mbar	CH <sub>4</sub> (?) (ref. 20)	CH <sub>4</sub> (?) (ref. 20)
Temperature variations (peak-to-peak) at $p \sim 10^{-3}$ mbar	35–45 K*	5–25 K (refs 25, 26)*
Origin of temperature variations	? (ref. 21)	? (ref. 21)

\* Maximum value, see text for discussion

The thermal structure of Uranus is somewhat different from that of Neptune, as is shown by the summary of IR and occultation temperatures in Table 1. Both planets have nearly the same effective temperatures, and this has been interpreted as showing that Neptune radiates about 2.8 times the energy it receives for the Sun, while Uranus radiates no more than 1.23 times the energy than it receives<sup>22,23</sup>. If Uranus has any internal heat source at all, it must be a smaller fraction of the solar energy received than the internal heat sources of the other giant planets<sup>23,24</sup>. The temperature inversion between the regions probed by IR and occultation measurements is much greater for Neptune than for Uranus. This is consistent with the conclusions of Gillett and Rieke<sup>17</sup>, who found a stronger temperature inversion for Neptune on the basis of methane and ethane emission at 8 and 12  $\mu$ m.

Both planets show temperature variations in the region probed by occultations. However, the cause of the variable temperatures and their relationship to radiative processes occurring in the upper atmospheres of these planets remain unknown.

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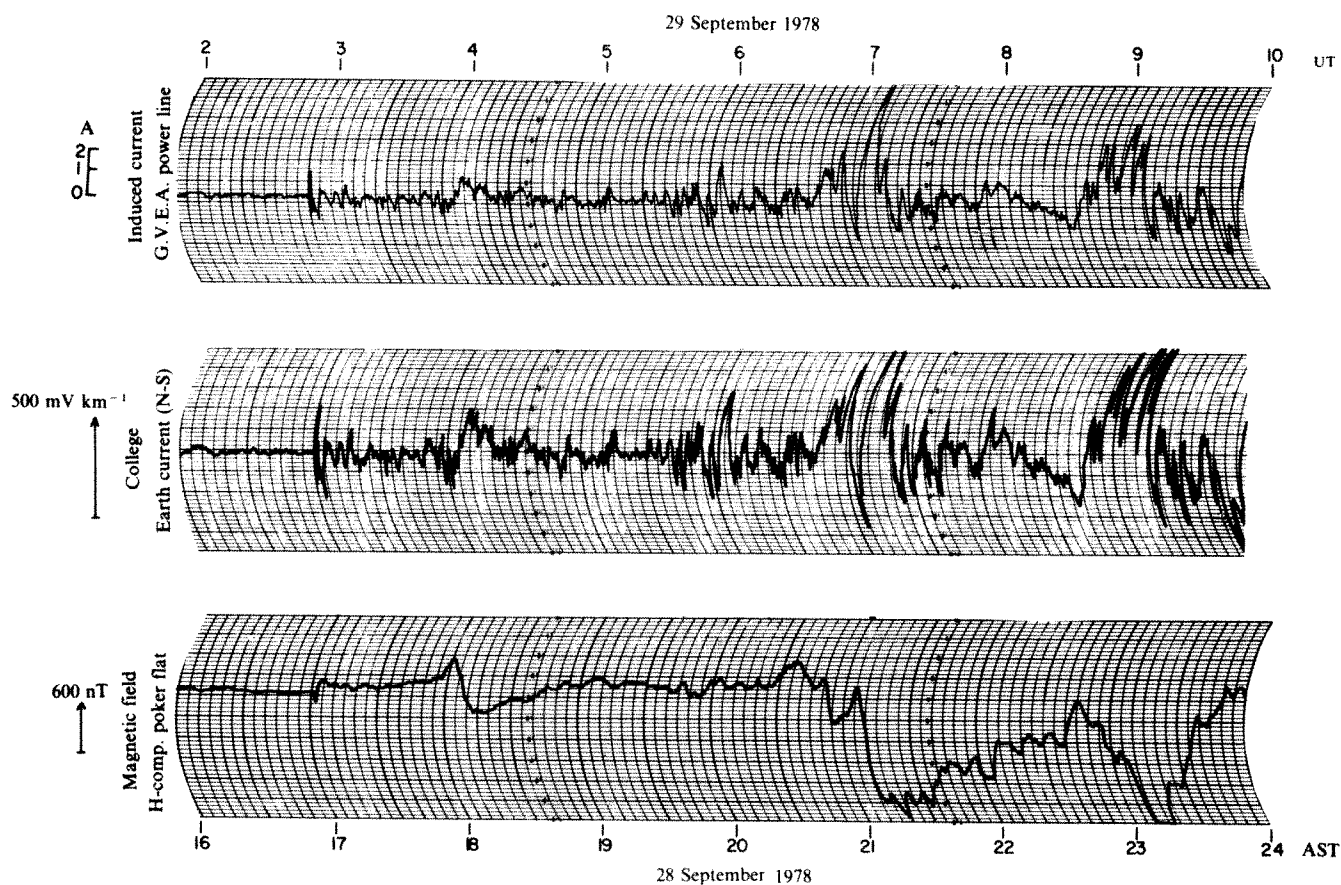
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## Electric currents in power transmission line induced by auroral activity

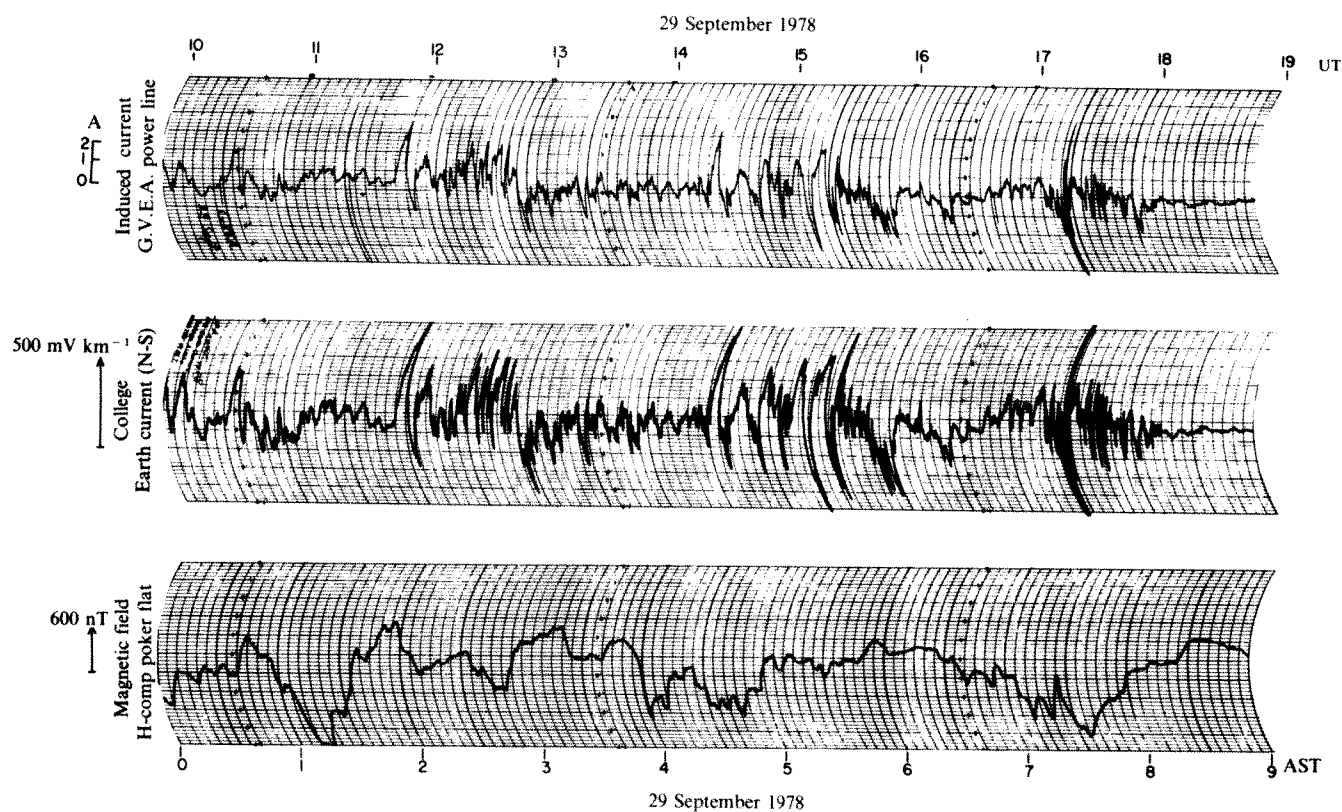
SURGES of electric currents induced by auroral activity were first observed in the middle of the last century in the wires of the electric telegraph<sup>1,2</sup>. In more recent years, auroral effects on long conductors, such as electric power transmission systems, telephone cables and oil pipelines, have attracted much attention because of various problems caused by these currents<sup>3</sup>. We report here measurements of the currents in a local power transmission line between Healy and Fairbanks and show that some of the fluctuations are aurorally induced.

The electric currents (induced by auroral activity) are caused by transient geomagnetic field variations which arise from concentrated ionospheric currents, called the auroral electrojets. Since the Earth is a spherical conductor, during intense auroral activity transient magnetic variations caused by the growth and decay or a rapid movement of the electrojet produce an induced Earth–surface potential. The induced potential in turn produces currents in a conductor, such as power, communication or pipeline systems, that are grounded to the Earth at two separate points<sup>4,5</sup>.

This induction effect is greatly complicated by various factors, such as the geometry of the conductor system with respect to the auroral electrojet and the geological structure in the vicinity of the conductors<sup>6</sup>. These effects are difficult to quantify in modelling the induction effect. Thus, to understand effects of the induced currents on powerline systems, the magnitude of the current must first be determined experimentally and the relationship between characteristics of geomagnetic variations and the induced currents in power line systems examined. However, no previous report has demonstrated clearly that some of the disturbances in power transmission lines are caused by auroral activity on the basis of simultaneous power line records and auroral activity records.



**Fig. 1** Comparison of the induced current in the Healy-Fairbanks power line transmission with the north-south component of the Earth current monitored in the same vicinity, and the **H** component magnetic records; 02.10 UT, 29 September, 1978 (16.24 AST, 28 September, 1978); AST, Alaska standard time.



**Fig. 2** As for Fig. 1; 10.19 UT, 29 September, 1978 (0.9 AST, 29 September, 1978).

We have therefore begun to monitor the induced current in a local power transmission line (138 kV, 100 A) in the vicinity of Fairbanks, Alaska, along a grounded neutral transmission line between Healy (the generator site) and Fairbanks, of length of 166 km.

Figures 1 and 2 show both the induced current in the Healy-Fairbanks transmission line at one of the transformer sites (substations) in Fairbanks. We show here the simultaneous **H** component magnetic record and the induced electric field (commonly referred to as 'the earth or telluric current') record taken in Fairbanks as indicators of auroral activity and the auroral electrojet. By comparing the induced current in the Healy-Fairbanks transmission line and the Earth current record it is obvious that the fluctuating currents in the transmission line were induced by the auroral induction. The corresponding negative excursions in the **H** component magnetic record indicate that auroral substorms were in progress during the intense indication events (see ref. 7 and note also that the induction at 03 UT was caused by the sudden commencement of the storm of 29-30 September, 1978).

In the Fairbanks area, multiple ground points exist, making the measurement of the total induced current difficult. By developing a model of the grounding network, however, we have been able to determine approximately the total current flowing in the Healy-Fairbanks line from our measurement at the substation. On the basis of our measurement and the model study, it is indicated that excursions of  $\pm 4$  A recorded at the substation would represent  $\pm 8$  A d.c. flowing in the transmission line.

The voltage and current induced in a transmission line are proportional to its length. In spite of the severity of magnetic disturbances in the auroral zone, the Healy-Fairbanks transmission line has escaped major troubles from the induction effects in the past because of its relatively short length. A transmission line across the auroral zone of length more than 500 km will require a careful design to overcome the difficulties caused by the induced currents.

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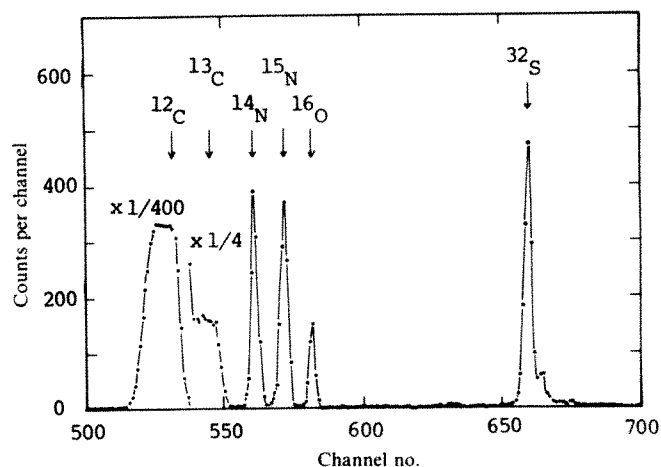
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## Analysis for $^{15}\text{N}$ by proton scattering

STABLE isotopes have a great potential as tracers in the life sciences and environmental research, but their uses are still limited compared with those of radioisotopes. Radioisotopes have been widely used as trace elements, although there are problems in handling radioactive materials and preventing environmental contamination. The increasing concern about exposure to small amounts of radioactivity has caused us to consider the advantages of stable isotopes. The detection of stable isotopes, however, is not as sensitive as that for radioactive tracers and the preparation of a sample for use in stable



**Fig. 1** Typical spectrum of protons scattered by  $^{15}\text{N}$  in methionine-enriched 95% in  $^{15}\text{N}$ .  $^{15}\text{N}$ -methionine was suspended in the 1/20-diluted *Physarum* cell to  $5 \text{ mg ml}^{-1}$ . The target backing in a diameter of 12 mm was coated with  $5 \mu\text{l}$  of the specimen. The target was bombarded at 6.70 MeV with an exposure of  $37.9 \mu\text{C}$ .

isotope analysis is complicated. We have looked at the possibility of using a nuclear reaction caused by accelerated charged particles as a way of detecting stable isotopes. We have developed a system of stable isotope analysis using proton elastic scattering. Elastic scattering was chosen because the cross-sections are generally an order of magnitude larger than that of other reactions. Moreover, proton elastic scattering analysis has many advantages which are absolute, multi-element, rapid and subject to automatic data handling. Using this method an analysis for  $^{15}\text{N}$  has been done for biological studies. Nitrogen is an important component of various organic compounds which occur naturally but it has no long-lived radioactive isotopes. Here we outline the new system, and give an example of  $^{15}\text{N}$  analysis.

A system for  $^{15}\text{N}$ -labelled compounds has been developed using proton beams of the Tandem accelerator at the Research Center for Nuclear Science and Technology, the University of Tokyo. A clear peak of  $^{15}\text{N}$  in the energy dispersion of elastically scattered protons must be obtained for even a small quantity of the isotope. For this, an optimum experimental condition of incident energy, scattered angle, beam collimation, solid angle subtended by detector and pulse height analysis system, especially, pile-up rejection system must be found. Incident proton energies were selected on the basis of the excitation functions<sup>1,2</sup> of  $^{15}\text{N}(\text{p}, \text{p})^{15}\text{N}$  and of  $^{16}\text{O}(\text{p}, \text{p})^{16}\text{O}$ . Because  $^{16}\text{O}$  concentrations in natural biological samples are three orders of magnitude higher than that of  $^{15}\text{N}$ , the peak of the elastically scattered protons by  $^{16}\text{O}$  is too high and the tail of the  $^{16}\text{O}$  peak masks the peak of  $^{15}\text{N}$ . Thus, incident energies with low cross-sections for  $^{16}\text{O}(\text{p}, \text{p})^{16}\text{O}$  have been selected using the excitation function data<sup>2</sup>. In addition,  $^{15}\text{N}(\text{p}, \text{p})^{15}\text{N}$  excitation function was also compared to avoid undesirably low yield of the proton by  $^{15}\text{N}$ . We have therefore chosen several incident energies, 5.30, 5.82, 6.00, 6.30, 6.40 and 6.70 MeV, and have obtained spectra with test samples as described in the legend to Fig. 1. The incident energies of 5.82 and 6.70 MeV were selected because of a higher yield of the protons from  $^{15}\text{N}$  at 5.82 MeV and because of the clear separation of  $^{15}\text{N}$  peak from  $^{16}\text{O}$  at 6.70 MeV.

We used a scattering chamber with a Si solid-state detector as the detection system for elastic scattering protons. The detector was placed 191 mm away from the target and at  $165^\circ$  to the beam direction. Although an angle of  $180^\circ$  would be desirable for sufficient energy separation to resolve  $^{15}\text{N}$  peak from  $^{16}\text{O}$  peak, a scattering angle of  $165^\circ$  was selected because of geometrical limitation. The defining slit of the detector was 9 mm in diameter and the solid angle subtended by the detector was  $1.74 \times 10^{-3} \text{ sr}$ . To prevent background counts due to an



accumulation of proton pulses from  $^{12}\text{C}$  or  $^{13}\text{C}$  involved in backing materials, a 'pile-up inspector' was used. The scattered proton spectrum was recorded using a pulse height analyser. Backing material should not contain any element heavier than N, since protons scattered by heavier elements could easily result in increased background counts in lighter mass region. Thus, carbon foil, polyethylene and polypropylene films are chosen because of the thickness of available films and their constituent elements of carbon and hydrogen. Polyethylene films were normally used because they are thin (10  $\mu\text{m}$  thick), commercially available at a low cost, and samples can be easily spread on them.

Plasmodium of *Physarum polycephalum* were used for testing as it was being incubated in our laboratory<sup>3</sup>. Microplasmodia were incubated with semi-defined or synthetic medium<sup>4</sup>. The microplasmodia were collected by centrifugation, washed twice in water and diluted with water to a concentration of 1/20–1/10. The microplasmodia were then sonicated with an ultrasonic sonicator. The sonicated sample, volume 5–20  $\mu\text{l}$ , was dropped onto the backing by pipette, spread homogeneously on the backing material and dried.

Standard samples were prepared to compare yield ratios for the proton elastically scattered by  $^{15}\text{N}$  with respect to given concentrations of  $^{15}\text{N}$ -methionine. DL-methionine, enriched to 95% in  $^{15}\text{N}$ , was used. The  $^{15}\text{N}$ -methionine was suspended in sonicated microplasmodia which were prepared from culture on nutrient medium, and spread on polyethylene backings. Suspension in the sonicated microplasmodia was necessary because the methionine suspended in water could not be spread homogeneously onto the backing.

A typical spectrum is shown in Fig. 1. The peak of  $^{15}\text{N}$  was clearly separated from that of  $^{16}\text{O}$  or of  $^{14}\text{N}$ . The peak of  $^{32}\text{S}$  appeared clearly, which consisted of protons mainly from those in the methionine added. The spectra of  $^{12}\text{C}$  and  $^{13}\text{C}$  were dominated by protons from those isotopes in the polyethylene backing. The peak of  $^{14}\text{N}$  was due to protons mainly from  $^{14}\text{N}$  in crude lysate of the cell. The  $^{14}\text{N}$  counts were used as normalisation factor after the correction due to the contribution from  $^{14}\text{N}$  in the added methionine. Counting ratios of  $^{15}\text{N}/^{14}\text{N}$  are plotted as a function of  $^{15}\text{N}$  concentrations in Fig. 2. A linear relationship holds between  $^{15}\text{N}$  yields and added  $^{15}\text{N}$  concentrations.

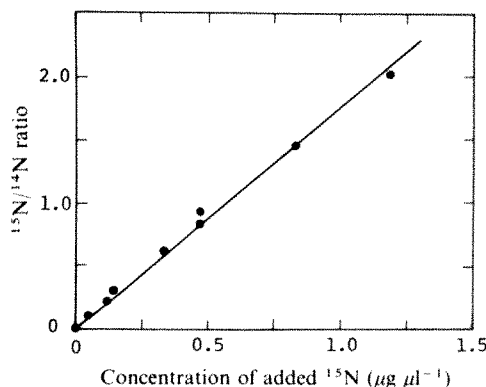


Fig. 2 A linear relationship between proton yields and  $^{15}\text{N}$  concentrations.

An example of a proton spectrum for a sample containing  $^{15}\text{N}$ -labelled compound is shown in Fig. 3. The sample was prepared from microplasmodia of *Physarum*, which had been incubated in the defined medium<sup>4</sup> supplemented with  $^{15}\text{N}$ -methionine (95% in  $^{15}\text{N}$ ). Twice washed microplasmodia were sonicated and spread onto the polyethylene backing. The specimen was bombarded at 6.70 MeV with a total exposure of 59  $\mu\text{C}$ . The separated peaks of  $^{14}\text{N}$ ,  $^{15}\text{N}$  and  $^{16}\text{O}$  appeared clearly. Peaks corresponding to  $^{23}\text{Na} + ^{24}\text{Mg}$ ,  $^{31}\text{P} + ^{32}\text{S}$ ,  $^{35,37}\text{Cl}$  and  $^{39}\text{K} + ^{40}\text{Ca}$  are also discernible.

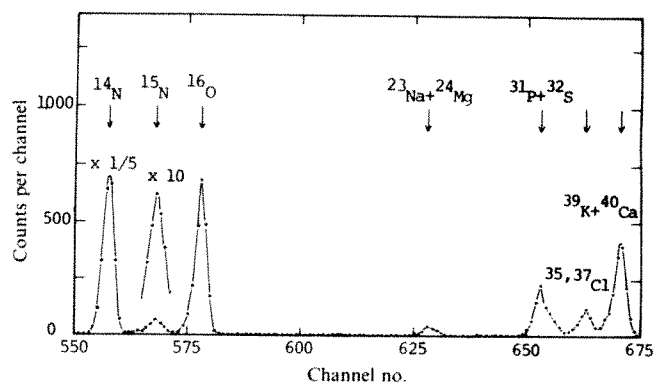


Fig. 3 Typical spectrum of protons by a bombardment of *Physarum* cell incubated with  $^{15}\text{N}$ -methionine.

The present method has advantages in that it can easily compare the abundance ratio of several elements and easily prepare samples. Even with a natural concentration of  $^{15}\text{N}$ , a peak of  $^{15}\text{N}$  was discernible and analyses for such samples are thought to be possible. However, to save time the solid angle of the detector must be increased. Further work is in progress to improve the system and to apply the system to the  $^{18}\text{O}$  analysis of biological samples.

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## Evidence for the presence of Freon 21 in the atmosphere

MONITORING of trace constituents of the atmosphere is becoming increasingly important because of the implications on the quality of life of growing concentrations of several compounds which, after industrial use, are released to the atmosphere. In particular, concentrations of Freons and other halocarbons need to be checked because of the problem of ozone depletion<sup>1–4</sup>. Recently, the search for tropospheric sinks of Freons has been considered in connection with the controversy about withdrawing such compounds from the market. The hypothesis that F21 ( $\text{CHCl}_2\text{F}$ ), which has no industrial use and apparently is only produced as an impurity of F12 ( $\text{CCl}_2\text{F}_2$ ), could be a decomposition product of F11, has been made by several workers<sup>5,6</sup>. However, detection and quantitative analysis of this compound in the atmosphere is extremely difficult because: (1) it is present in extremely low concentration<sup>7</sup>; (2) gas

chromatography (GC) separation from other halocarbons is difficult<sup>8</sup>; (3) there are several interferences and sample contamination may easily occur. The presence of F21 in the atmosphere, although detected in several researches, is therefore still uncertain. Moreover, due to the sampling techniques used, which do not use strong sample concentration, to our knowledge no GC-mass spectrometry experiments have been reported. We report here the results obtained using GC-mass fragmentography for the analysis of F21 in atmospheric samples of country areas in the regions of Urbino and Rome.

The sampling technique, the elimination of artefacts due to impurities present in the carrier gas and trapping efficiency have been described elsewhere<sup>9</sup>. Between 5 and 20 l of air were passed through a stainless steel trap containing Carboxpack B, a graphitised carbon black (80–100 mesh), kept at the temperature of dry ice (about –90 °C), so that the break-through volume of F21 and other lighter halocarbons was higher than the amount of air sampled.

The GC was equipped with an oven where the trap was placed and heated to 200 °C. Injection into the column was by means of the heating-stripping technique, described elsewhere<sup>9</sup>.

Our major problem with the halocarbon analysis was to devise a suitable column to obtain a complete separation of all these compounds, for the reasons stated above. In particular, the separation of F21 from CH<sub>3</sub>I is important for the determination of the F21 concentration in the atmosphere. The column used for this purpose consists of Carboxpack B 80–100 mesh coated with 0.5% SP1000, a polar acidic liquid phase. This choice was made for two reasons. First, we use the outstanding selectivity of gas-liquid-solid chromatography, where the driving force of the chromatographic process is molecular polarisability, which in several cases is independent of the vapour pressure. Second, the modifications induced by rather low amounts of a polar liquid phase do not affect the selectivity towards non-polar compounds such as halocarbons.

Furthermore, by choosing an appropriate amount of liquid phase, the separation factors may be adjusted to the particular analysis required.

Rasmussen *et al.*<sup>9</sup> have reported the analysis of halocarbons trapped from 30 l of air in a suburban zone, showing that F21 is well separated from all the other halocarbons.

A major difficulty of quantitative analysis of halocarbons in the atmosphere is that some of these compounds are contained as ultra-trace impurities in high purity commercial gases. This problem has been solved by further purification of the carrier gas using a dry ice-acetone trap, and by purging the sampling traps with the purified gas before use.

Experiments have also been performed to see whether F21 could be an artefact due to sample treatment before GC analysis. A mixture of all C<sub>1</sub>–C<sub>2</sub> halocarbons except F21 was inserted in the trap and stripped at 170 °C. The resulting chromatogram did not show any peak with the retention time assigned to F21.

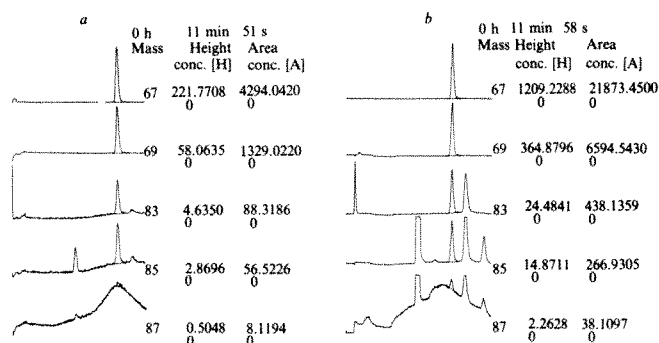
Mass fragmentography was performed using a VG/MM 305 mass spectrometer equipped with a jet separator and a data acquisition system.

Values of *m/e* 67, 69, 83, 85, 87, were monitored and the relative abundances of these fragments were found to be consistent with the mass spectrum of F21.

An accurate determination of the atmospheric concentration of F21 is not yet possible. However, it has been estimated to range between 10 and 20 p.p.t. v/v.

**Table 1** Relative concentration of F21 in the atmosphere of Rome and Urbino from GC data

Sample no.	Location	Sample vol (l)	Peak area (cm <sup>2</sup> )	Electrometer attenuation	Relative concentration
1, 2, 3, 4	Urbino	20	1.34	×512	1.00
5, 6, 7	Urbino	10	1.62	×256	1.20
8, 9, 10	Rome	10	1.20	×256	0.97
11, 12, 13	Rome	5	0.57	×256	0.85



**Fig. 1** Mass fragmentograms of a reference sample of F21 (a) and of the peak with the same retention time in an actual air sample (b). Computer output of the data. For details see text.

Table 1 shows the relative concentrations of F21 found in different samples from several locations. For each location the peak area is the arithmetic mean of the measurements made. Measurements within each location scatter about 20%, which is about the same as that found among measurements in different sites. Thus, it can be stated that, within the experimental errors, the concentration of F21 is fairly constant.

The computer output of the GC-MF analysis of one of the atmospheric samples is shown in Fig. 1: (a) refers to the standard F21 and (b) to the peak with the same retention time in an actual mixture of halocarbons trapped in the Urbino environment (20 l of air).

Fifteen samples trapped both in Rome and in Urbino have been analysed by GC-mass fragmentography and F21 was found to be present in each sample. The results of these experiments show that F21 is actually present in the atmosphere and cannot be considered as an experimental artefact.

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## India and Madagascar in Gondwanaland based on matching Precambrian lineaments

THE geometrical fit of Madagascar against India has been widely accepted in most reconstructions of Gondwanaland<sup>1</sup>. This fit is consistent with Precambrian trends, lithologies and age provinces<sup>2,3</sup>. Palaeomagnetic data also support the general fit of Madagascar against India<sup>4</sup>, and there are suggestions that this reassembly can be recognised as far back as Proterozoic times<sup>5</sup>. The exact fit of east Madagascar and west India has not been adequately defined. Recent knowledge of the Precambrian geology and tectonics, has now allowed the possibility of better defining this fit, mainly on the basis of matching Precambrian re-entrant lineaments. The east coastline of Madagascar is a

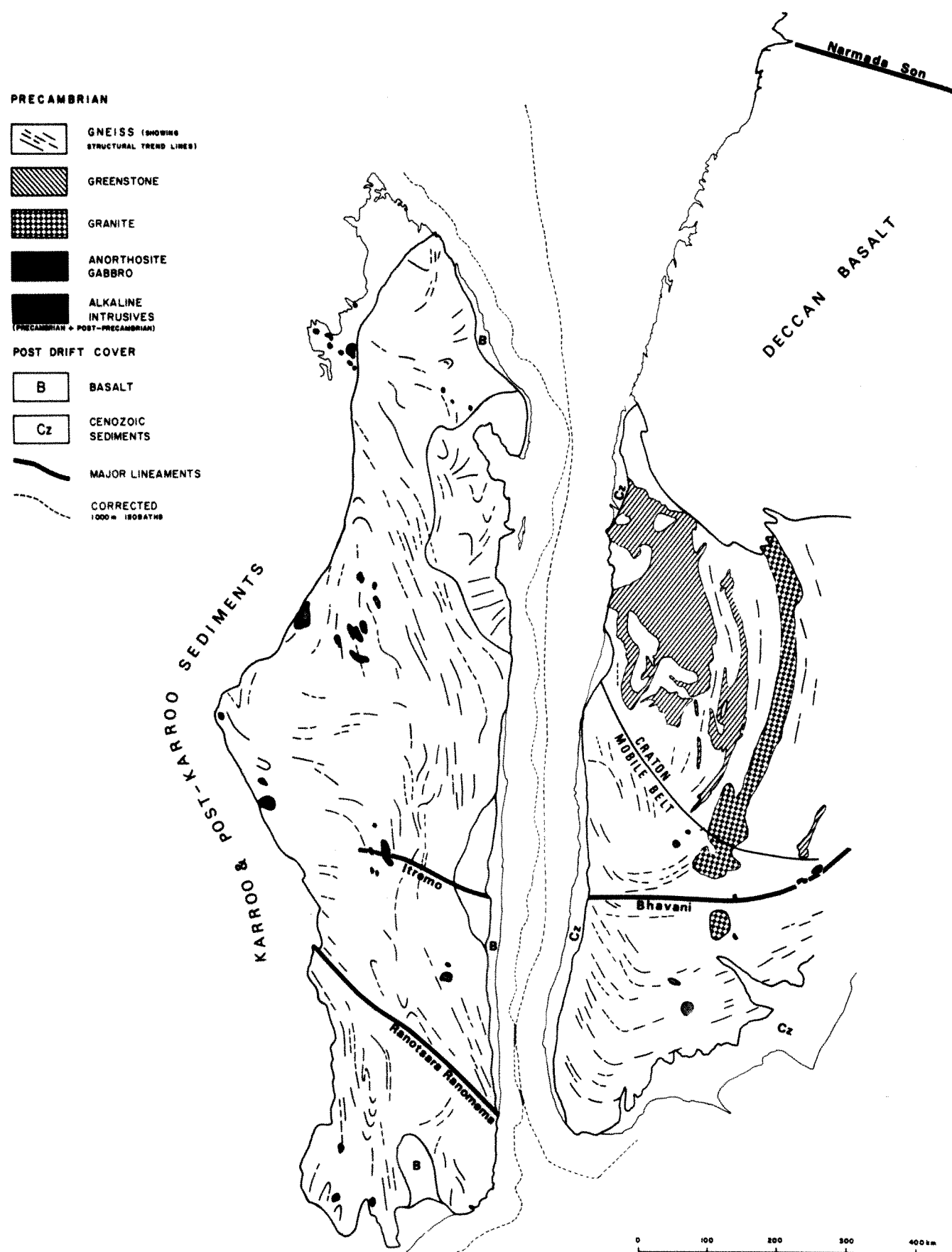


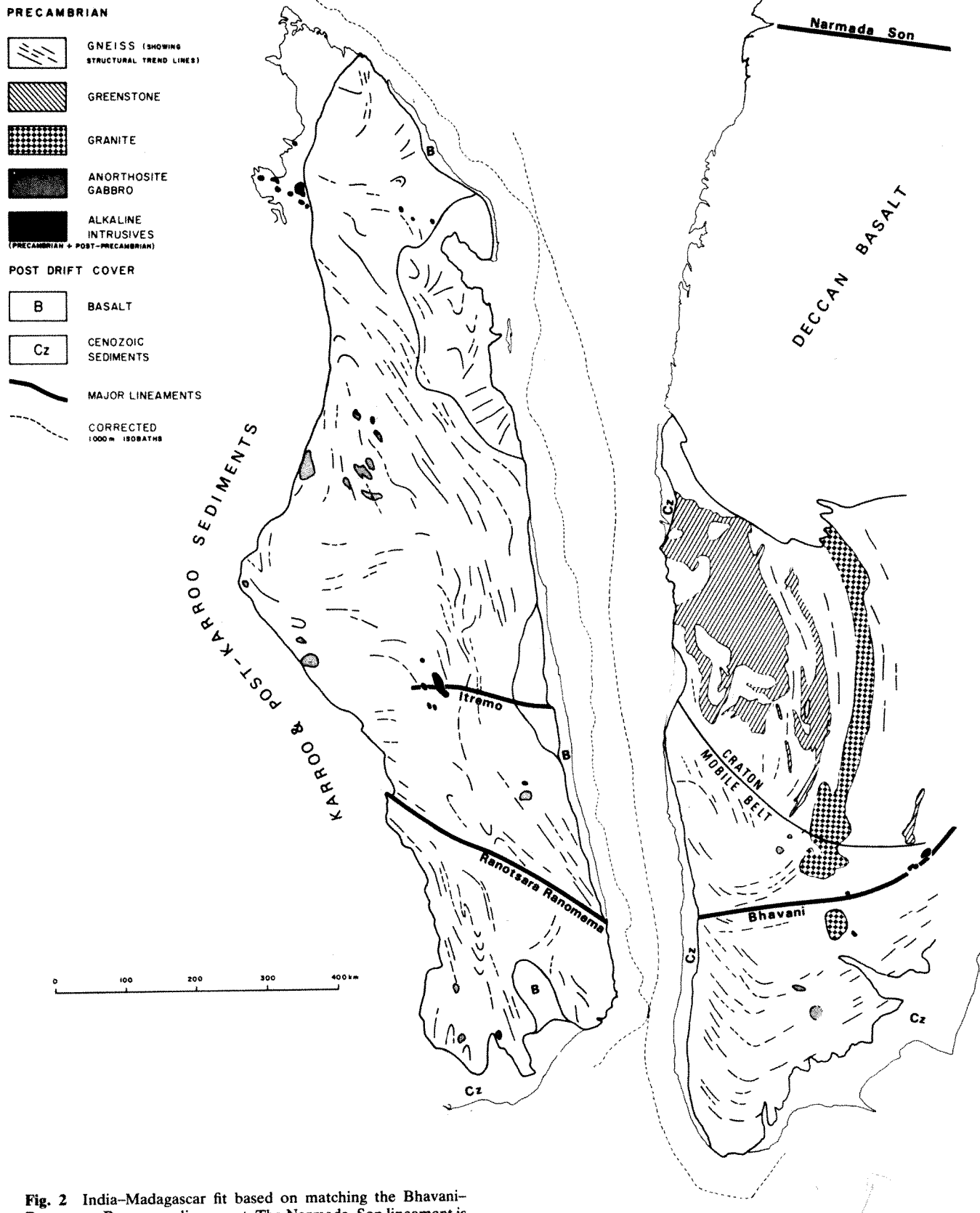
Fig. 1 India-Madagascar fit based on matching and Bhavani-Itremo lineaments. Areas in Madagascar outlined on the east coast are dated as Archaean and could possibly represent a craton.



rectilinear feature, with a narrow shelf of 25–50 km and an abrupt steep break in slope after the 1,000-m isobath. The proposed opposing coastline of west India is also rectilinear with a wider shelf of 100 km and a shelf break down to the 1,000-m isobath. The 1,000-m isobath of India was receded towards the coastline by removing the post drift sediments, according to the

best available sections<sup>6</sup>. A 1:2,500,000 conical projection was used to prepare a distortion-free, equidistance base map of both Madagascar and India (Fig. 1).

The Archaean terrain of south India consists of a craton, greenstone–gneiss complex surrounded by a charnockite mobile belt<sup>7</sup>. Basement gneisses have been dated as old as 3,200 Myr



**Fig. 2** India-Madagascar fit based on matching the Bhavani-Ranotsara-Ranomema lineament. The Narmada-Son lineament is continuous with a new lineament in northern Madagascar, shown here.

and old greenstones in the craton are about 3,000 Myr. Charnockite metamorphism in the mobile belt has been dated at about 2,800 Myr. Younger greenstones in the craton are about 2,600 Myr, and the granites have been dated at about 2,500 Myr. Proterozoic dates recorded in both the craton and mobile belt reflect later periodic events at about 2,000 Myr, 1,600 Myr and 900–600 Myr. The Archaean of Madagascar consists of the charnockitic complex of southern Madagascar, and a patchy distribution along the east coast<sup>8,9</sup>. The widely distributed Archaean dates suggest that most Precambrian Madagascar has an Archaean history. A strong Proterozoic overprint has masked this proposed widespread Archaean event. In contrast to India, no cratonic greenstone complexes are recognised and most of the rocks appear to belong to a charnockitic mobile belt<sup>8</sup>. The observation that the Indian craton is girdled by a charnockitic mobile belt seems substantiated by the evidence emerging from the proposed Madagascar–Indian relationships in Precambrian Gondwanaland. The only regional lithological correlations between south India and Madagascar are a charnockitic–gneiss mobile belt containing many anorthosite–gabbro bodies, that sweep around the southern and western margins of the Indian craton. The structural trend lines of the mobile belt recognised in south India can be traced into its equivalent in Madagascar<sup>2,3</sup> (Fig. 1).

From the economic viewpoint neither south India nor Madagascar are intensely mineralised. The paucity of mineral deposits recorded is not a conclusive indication of all overall poor mineral potential, as the tropical rain forest conditions and the rather attenuated exploration efforts in the past have undoubtedly contributed to a poor mining development. The metallogenesis of most of south India and Madagascar is restricted to silicates and oxides related to their common mobile belt tectonic setting. Similarly, the essentially gold–sulphide mineralisation of the Indian craton are not significantly represented in Madagascar. Several small mineral occurrences are known and occasionally exploited, and their consistence appears to be more than coincidental. Among the most obvious are the marine beach placers of monazite, zircon, ilmenite and rutile derived from the hinterland charnockitic massifs, the niobium–cerium mineralisation found associated with lineaments in Madagascar and south India, and the chromite, graphite, phlogopite and garnet occurrences.

Lineaments thought to penetrate the Indian–Madagascar parts of Gondwanaland have been used to define a fit<sup>10</sup>. The Narmada–Son lineament of northern India has been traced into northern Madagascar<sup>11</sup>. Other fundamental Precambrian lineaments recently described from south India and Madagascar can be utilised to define a more exact fit. In particular, the Bhavani lineament of south India<sup>12</sup> is compared to two important lineaments in Madagascar, the Itremo lineament of central Madagascar<sup>9,13</sup>, and the Ranotsara–Ranomena lineament of south Madagascar<sup>8</sup>. (Fig. 1). The fits described are compatible with the reconstruction models of Gondwanaland. The 1,000-m isobath of Madagascar is placed up against the receded 1,000-m isobath of India. This allows about 100 km of intervening continental shelf separating the opposing coastlines. Madagascar is translated relative to India along this line until the best fit is obtained in regard to geometry, bathymetry, age and lithological, structural and tectonic relationships. To define the tectonic line of reference, the Bhavani lineament was chosen. This lineament is a re-entrant feature from the Indian coastline and can be followed in a curvilinear trace to the north-east for about 500 km. The Bhavani lineament has been recognised as having an important role in the Precambrian tectonic evolution of south India<sup>12</sup>, and also controls the emplacement of alkali intrusives, including carbonatites and ultrabasics<sup>14</sup>. A similar lineament has been described from central Madagascar, here termed the Itremo lineament<sup>9,13</sup>. Several alkaline intrusions have also been recognised along the Itremo lineament. If the Itremo–Bhavani lineaments are matched, a penetrative, curvilinear feature can be traced across the Indian–Madagascar mobile belt (Fig. 1). Another possible fit is to match the Bhavani

lineament with a more distinctive lineament in south Madagascar, termed the Ranotsara–Ranomena lineament<sup>8</sup> (Fig. 2). The Ranotsara–Ranomena lineament is a major discontinuity separating the southern charnockitic block from the gneiss terrain to the north. The matching of the Bhavani–Ranotsara–Ranomena lineaments also satisfies many of the geological criteria set out above.

There is not yet enough information to establish, with certainty, which fit is best. If the Bhavani–Itremo fit is selected, it defines a major lineament which apparently controlled the emplacement of alkali intrusives and carbonatites. This fit also satisfies the geometrical and bathymetrical relationships. The Ranotsara–Ranomena lineament on extension into India can control continental margin features on the south coastline of India (Fig. 1). On the other hand, the Bhavani–Ranotsara–Ranomena fit also satisfies similar criteria. The Itremo lineament could still extend into India, as similar tectonic lines have been recognised from LANDSAT as re-entrants from the coast of west India<sup>15</sup>. In addition, the Narmada–Son lineament of northern India lines up with a new lineament recognised in northern Madagascar<sup>9</sup> (Fig. 2). Both of these reconstructions define major, penetrative curvilineaments that could have controlled small circle, prototransform directions in the Indian–Madagascar mobile belt, that contributed to the rifting of India from the Madagascar–African plate in Cretaceous times. The position of rifting also indicates possible control by the structural trends of the mobile belt and the presence of granulite facies charnockitic rocks<sup>16</sup>.

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## Short-term climate change and New Zealand temperatures during the last millennium

MANY theories have been proposed to explain the climatic fluctuations which produced the sequence of glacial–interglacial periods of the order of tens of thousands of years which have occurred throughout the Quaternary. These fluctuations represent temperature changes in the temperate regions of about 6 °C between a glacial and an interglacial situation. There are also climatic fluctuations with a period of the order of about a thousand years representing temperature changes of about 2 °C. These changes are believed to have had an important bearing on human history, but have been little studied because it is difficult to obtain accurate temperature records for past

periods. Shorter temperature fluctuations of the order of a few decades, and representing temperature changes of perhaps as much as one degree, are of considerable economic importance and merge into the developing field of long-term meteorological forecasting. Considerable interest has recently developed in short-term climate changes. If detailed long-term climatic records could be obtained for extended periods in the past it might be possible to determine the causes for the climatic variation, and hence predict future climate trends. Even a knowledge of how hot/cold or dry/wet a particular region's climate could become, and with what probability, would be of considerable economic value to planners involved in hydro-electric development, irrigation schemes, snow clearance and the development of arid and polar regions. At present instrumental records have existed only since the mid-seventeenth century for central England, and for most other areas only over a little more than a century. Cave deposits (speleothems) provide stratigraphy which has a high inherent time resolution. Data for studying short-term temperature fluctuations can be obtained by measuring the  $^{18}\text{O}/^{16}\text{O}$  ratio of suitable stalagmites<sup>1</sup>. This technique should enable a high resolution temperature curve to be produced for many regions of the globe. We report here an investigation on the  $^{18}\text{O}/^{16}\text{O}$  profile through a New Zealand stalagmite which was undertaken partly to evaluate the feasibility of obtaining high time resolution data from speleothem material and partly to compare the temperature record from New Zealand (in the Southern Hemisphere and a region meteorologically unrelated to Europe) with the English climate curve, which is the most firmly established climate curve for the last millennium. Such proxy data given us the possibility of obtaining long-term high resolution temperature records from some of the critical regions of the world where meteorological records either do not exist or are of very short duration.

Long-term climatic fluctuations such as glacial-interglacial sequences have been studied by measuring the oxygen isotopic ratios in the carbonate from the foraminifera found in deep sea cores (see, for example, ref. 2). The movements of bottom living organisms and low sedimentation rates, however, limit the time resolution obtainable from most of these cores to about 3,000 yr. It may, however, be possible to carry out similar work in suitable regions of very high sedimentation rate and to obtain data for studies of short-term temperature fluctuations at least in more recent times.

Tree rings are another possibility for obtaining high resolution palaeoclimatic information (see, for example, ref. 3). Dendrochronology provides us with stratigraphic sequences, sometimes of considerable length, which can be obtained from many parts of the world. The bristlecone pine chronology from the White Mountains in California, for example, has a length of some seven millennia<sup>4</sup>. The isotopic-geochemical problems are formidable but a time resolution better than one year and absolute dating system accurate to one year could be obtained.

The work described here exploits the stratigraphy present in cave deposits where the chemistry is simple but the dating is less certain than with tree rings. It may eventually be possible to combine information from both sources and use the tree-ring record to confirm and correct the speleothem time scale.

If it can be assumed that a stalagmite has been laid down in isotopic equilibrium with the water then the oxygen isotope ratio of its calcite is controlled by two factors<sup>1</sup>: (1) the isotopic composition of the water flowing into the cave; and (2) the temperature at which the calcite is deposited on the speleothem, which for many caves can be taken as the mean annual temperature. The isotopic fractionation of the oxygen isotopes between calcite and water will increase by 0.024‰ as the temperature of deposition decreases by 1°C. As existing mass spectrometric techniques enable relative measurements of  $^{18}\text{O}/^{16}\text{O}$  to be made to 0.0025%, one should be able to obtain palaeotemperature curves to 0.1°C and this should be useful for studying short-term climatic fluctuations.

The  $^{18}\text{O}/^{16}\text{O}$  composition of the atmospheric precipitation percolating into the cave is determined first by the isotopic

composition of the oceans. This changes between the glacial and interglacial situation due to the removal of water depleted in  $^{18}\text{O}$  and its deposition on the ice sheets; however, this effect is negligible for the short-term temperature fluctuations in the present study. The second effect is caused by the temperature difference between the region of evaporation and the area under study. This effect tends to work in the opposite direction to the effect of temperature and hence would tend to reduce any fluctuations due to temperature. However, if tropical stalagmites are measured they should give temperature changes of the tropics directly. Further comparison between stalagmites from tropical and temperate regions will give a plot of changes in temperature differences during time and hence a record of past latitudinal temperature gradients. Such data could provide a measure of the intensity of the zonal atmospheric circulation in the past and throw light on such problems as loess deposition and sand dune formation and even provide interesting archaeological information.

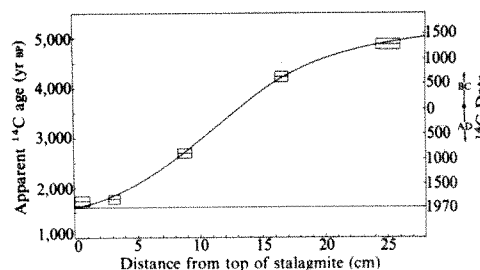
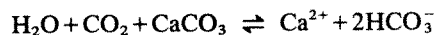


Fig. 1  $^{14}\text{C}$  data from which the stalagmite was dated. Horizontal bars represent areas of the stalagmite sampled. Vertical bars represent  $\pm$  one standard deviation of the  $^{14}\text{C}$  determination.

Gathering any palaeoclimatic data from stratigraphy involves some dating method. In the case of speleothems this can be achieved for the past 35,000 years by  $^{14}\text{C}$  dating. However, the carbon laid down as carbonate on a stalagmite contains a mixture of ancient carbon from the limestone carbonate, which contains essentially no  $^{14}\text{C}$ , and modern carbon respired by plant roots. The calcium in solution has been dissolved from the limestone according to



Assuming that saturation is reached as the  $\text{CO}_2$  solution percolates through the limestone and into the cave then a little more than half the carbon in the resultant solution would be of recent biogenic origin and a little less than half derived from limestone<sup>5</sup>. This theoretical situation is not always the case (for example, the Twin Forks stalagmite). This is due to the exchange of carbon dioxide with cave air. Hendy<sup>5</sup> has described how such corrections can be made using  $^{13}\text{C}/^{12}\text{C}$  ratios. In the case of a stalagmite which is still growing when collected, an even more accurate estimate can be made by determining the  $^{14}\text{C}$  content of the outer layer of the stalagmite. Other methods such as thermoluminescence are also potentially useful for dating a material such as calcite. Several samples must be dated across the stalagmite to correct for any variation in growth rate of the stalagmite during the time under investigation.

We examined a stalagmite from a cave in north-west Nelson lat  $40^\circ 40' \text{S}$ , long  $172^\circ 26' \text{E}$  and growing at an altitude of about 50 m above sea level. The stalagmite was taken near a meteorological station which has a present-day mean temperature of  $12.2^\circ \text{C}$  (ref. 6). The stalagmite was sectioned and 30-g samples taken for  $^{14}\text{C}$  determination to provide a time base. These data are presented in Fig. 1. Samples ( $\sim 50 \text{ mg}$ ) were also taken at regular intervals down the axis of the stalagmite for isotopic analysis with a 1.6-mm steel drill. Five 10-mg aliquots of each of the samples of calcite were reacted with 100% phosphoric acid at  $25.0 \pm 0.1^\circ \text{C}$  in evacuated glass reaction vessels. The carbon dioxide was purified<sup>7</sup> and the ratios of mass



45 to (mass 44 + mass 46) and mass 46 to (mass 44 + mass 45) were compared with a sample of carbon dioxide prepared from Te Kuiti limestone<sup>8</sup> on a Nuclides Analysis Associates 60° double collector mass spectrometer. The  $^{18}\text{O}/^{16}\text{O}$  ratio is reported as  $\delta^{18}\text{O}$  with respect to the international standard PDB<sup>8</sup>, where

$$\delta^{18}\text{O}(\text{‰}) = \frac{^{18}\text{O}/^{16}\text{O}_{(\text{sample})} - ^{18}\text{O}/^{16}\text{O}_{(\text{PDB})}}{^{18}\text{O}/^{16}\text{O}_{(\text{PDB})}} \times 1,000$$

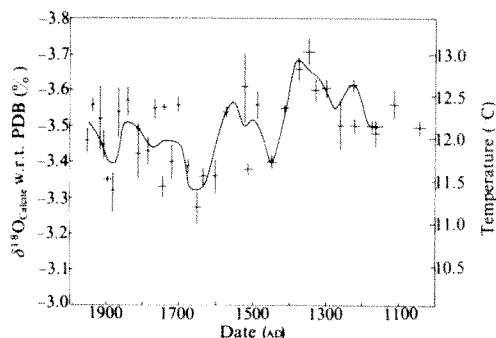
The mean values of the  $\delta^{18}\text{O}$  are plotted in Fig. 2, the error bars represent one standard deviation of each particular set of measurements. In such a study the  $^{13}\text{C}/^{12}\text{C}$  ratio must be plotted against the  $^{18}\text{O}/^{16}\text{O}$  ratio to ensure that they do not correlate and hence confirm that the stalagmite was laid down in conditions of isotopic equilibrium<sup>5</sup>. The results of these data are combined as a palaeotemperature curve on Fig. 3 and compared with Lamb's curve for central England<sup>9</sup>.

The curve has been scaled in terms of temperature as we know the temperature at which the stalagmite was growing when collected and the temperature change for the last cold period in New Zealand. This cold period occurred during the first decade of this century and was recorded by direct instrumental observations. As the above estimate agrees well with the known temperature dependence of the fractionation factor of calcite, it suggests that little or no change in the groundwater composition has taken place and the isotope fluctuations in the stalagmite studied seem to be mainly due to temperature changes.

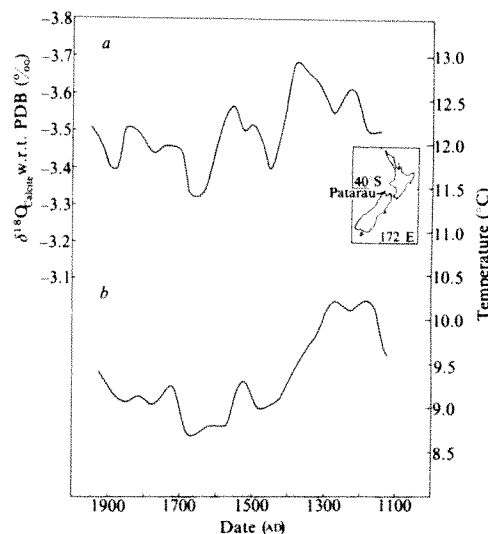
These very preliminary results mainly investigate the potential of stalagmites to study short-term temperature variations. Clearly, many stalagmites should be taken from different caves in different parts of New Zealand. However, the temperature curve for New Zealand is apparently broadly similar to England and such climatic fluctuations as the Mediaeval Warm Period and Little Ice Age are not just a local European phenomenon.

It seems from the temperature curve that the cooling in New Zealand has been delayed and is generally more rapid than in central England. This may be caused by dating errors. The temperature is known better than the actual date of the New Zealand curve whereas the date is known accurately from historical records but the temperature may be in error for the English curve. However, the past 100 yr of the curve agree well with New Zealand meteorological records<sup>10</sup> and not the English temperature curve during the same period. Whereas the temperature of England since the 1940s has fallen significantly<sup>13</sup> the mean New Zealand temperature is still climbing<sup>10</sup>.

An interesting feature of the curve is that in recent times samples representing times as short as 10 yr were measured. Because the hole drilled in the stalagmite is circular in section more calcite is obtained from the central years of the time span



**Fig. 2**  $^{18}\text{O}/^{16}\text{O}$  profile through the stalagmite expressed in ‰ deviation from the international isotope standard PDB (Pee Dee Belemnite). The temperature scale on the left has been estimated using the modern day mean temperature for the region from which the stalagmite came and the known temperature fractionation of the calcite water system. The error bars represent one standard deviation of each particular set of measurements. The solid curve is the 50-yr running mean.



**Fig. 3** The 50-yr running mean curve (a) from Fig. 2 is compared with that from central England (b) given by Lamb, ref. 9.

with progressively less as the extremes are approached. A limiting factor to the time resolution of the technique is that winter precipitation is more depleted in  $^{18}\text{O}$  than summer precipitation. If there is more winter growth than summer or vice versa the point will be displaced upwards or downwards respectively. This effect only becomes significant when a small number of years is sampled. The problem could be overcome by drilling out many samples along a growth line and mixing them. However, this study shows that the technique is capable of a time resolution of only a few years and is apparently limited only by the winter/summer sampling problem. As can be seen from the New Zealand temperature curve, the very rapid temperature drop in the fourteenth century may well have had a catastrophic effect on the Polynesian agriculture, based as it was on the tropical food plants (taro and kumera) in all except the very north of the North Island. It is probably no coincidence that the Polynesian exploration of the South Pacific came to an end at this time. As has been discussed in an earlier paper<sup>14</sup> a cooling might be expected to lead to steeper latitudinal temperature gradients and hence to a more violent climate. The loss of travellers by the increase in frequency of more violent storms would effectively discourage further exploration.

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## Effect of rapid precipitation of dissolved Mn in river water on estuarine Mn distributions

ALTHOUGH the general characteristics of estuarine chemical behaviour can be deduced from distributional data<sup>1</sup>, there is an urgent need for greater understanding of the mechanisms and kinetics of the processes involved to assist the development of precise geochemical cycling models and to allow accurate predictions of the consequences of waste disposal into estuaries and inland waters. Manganese is of particular importance in this respect because, as well as exhibiting pronounced reactivity within estuaries<sup>2-4</sup>, the highly absorptive properties of particulate and colloidal manganese oxide phases may contribute significantly to the estuarine behaviour of many other trace metals<sup>5</sup>. We show here how measurements of the effects of suspended particles and salinity on the rate of precipitation of dissolved manganese can lead to an explanation of the temporal and geographical variability in the distribution of dissolved manganese in an estuarine system.

During investigations of the distribution of manganese in the Tamar Estuary, South-west England, we have occasionally encountered a minimum in dissolved manganese (which can fall below  $1 \mu\text{g l}^{-1}$ ) in the freshwater immediately above the salt wedge. Such minima invariably coincide with high concentrations of suspended particulates produced by intrusion of the estuarine turbidity maximum into the freshwater. Upstream of the limit of this intrusion, dissolved manganese concentrations are usually within the range  $20\text{--}60 \mu\text{g l}^{-1}$ . These results indicate a rapid removal of dissolved manganese by the suspended particles. A study of this process has demonstrated zero-order reaction kinetics with respect to dissolved manganese in freshwater and has provided evidence of the mechanisms involved.

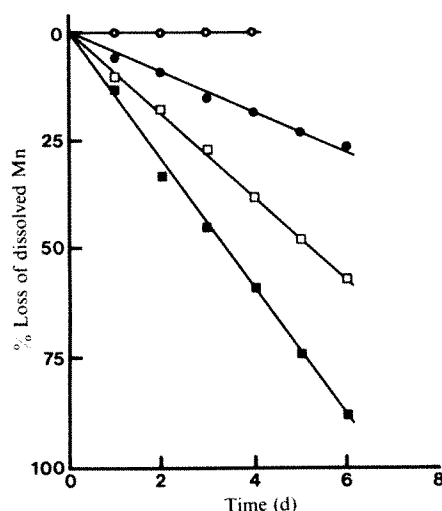
All experiments were carried out on samples of natural water obtained from the river and incubated in the dark at constant temperature. Dissolved manganese concentrations (passing a  $0.45 \mu\text{m}$  pore-sized membrane filter) were determined using the flameless atomic absorption procedure of McArthur<sup>6</sup> and the weight of suspended particulate material (dried at  $110^\circ\text{C}$ ) was measured gravimetrically. Experimental manipulation of suspended particulate concentration was achieved by mixing proportions of natural river water, collected either from within

or from upstream of the zone of maximum turbidity, with water which has been filtered through a  $0.45 \mu\text{m}$  membrane filter.

A rapid loss of dissolved manganese in natural river waters was obtained during these experiments which was kinetically of zero-order with respect to the concentration of dissolved manganese (see Fig. 1). In contrast, removal of dissolved manganese from estuarine waters approximated to first-order with respect to dissolved manganese and was considerably slower. The rate of removal decreased with increasing salinity. The zero-order rate law applied to samples of river water whether or not the particulate material originated from within the estuarine turbidity maximum, for particulate concentrations in the range  $0.5\text{--}500 \text{ p.p.m.}$ , and at temperatures within the range  $5\text{--}20^\circ\text{C}$ . Increasing temperature always accelerated the reaction. No detectable loss of manganese was apparent in samples from which the particulate materials were removed by filtration and the rate of removal increased with increasing particulate load. However, it was generally evident that lower particulate concentrations exerted a disproportionately large effect, particularly at higher temperatures. Furthermore, the rate of manganese removal varied significantly between experiments carried out at different times but with equivalent concentrations of particulate material. Using the Arrhenius equation to calculate the apparent activation energy of the removal process has consistently yielded low values, usually within the range  $5\text{--}15 \text{ kcal mol}^{-1}$ . The combined effects of temperature and the concentration of particulate material on the zero-order removal rate for a typical experiment are shown in Fig. 2.

Morgan's<sup>7</sup> extensive investigations would indicate that, for an oxidative reaction within an initially homogeneous solution corresponding in composition to the river waters under investigation, the reaction should, at first, proceed with first-order kinetics with respect to divalent manganese, but would be extremely slow. The oxidative reaction is, however, subject to pronounced autocatalysis generated by manganese oxide reaction products<sup>7,8</sup>. Other naturally-occurring particulate or colloidal surfaces, for example, silica<sup>9</sup>, feldspar<sup>10</sup>, hydrated aluminium and iron oxides<sup>5</sup>, seem to catalyse the oxidation although their catalysis may well be due to, or enhanced by, the presence of manganese oxide surface 'impurities'. Furthermore, manganese oxides, particularly if freshly precipitated, are highly efficient and to some extent specifically adsorptive and/or coprecipitative scavengers of certain metal ions, including divalent manganese<sup>11,12</sup>. Taking these properties into account, Crerar and Barnes<sup>13</sup> and Hem<sup>14,15</sup> have proposed that coupled specific adsorption-catalysed oxidation reactions occurring at the surface of pre-existing manganese oxide solids, with consequent regeneration of the reaction sites, can explain the continuous accretion of hydrogenous manganese oxide solid phases.

The kinetic results reported here are in accordance with such a mechanism. By analysing both dissolved and particulate manganese during a kinetic experiment, we have found that the reaction products are quantitatively retained by the particulate phase, although the kinetic results show that the catalytically effective property of the particles is conserved during the reaction. This agrees with the self-regenerating properties inherent in the mechanism noted above, and indicates that pre-existing manganese or mixed manganese-iron oxide phases occurring as superficial coatings on the particles act as self-maintaining catalytic sites for continued uptake of dissolved manganese. The indifference of reaction rate to the dissolved manganese concentration, that is zero-order kinetics, implies sorptive occupation of all available reaction sites by the reactant throughout the course of the reaction. This is possible if the rate of attainment of sorptive equilibrium for divalent manganese on the catalytic phase is considerably faster than the overall reaction rate. Experimental sorption studies<sup>11</sup> indicate that this is so. The slower, first-order reaction characteristic of estuarine waters can be attributed to a significant competition for occupation of the catalytic sites exerted by the major seawater cations.



**Fig. 1** Loss of manganese from solution in Tamar River water containing 0% (○); 10% (●); 50% (□); and 100% (■) of the original particulate load during incubation at  $5^\circ\text{C}$ . The water originally contained  $47 \mu\text{g l}^{-1}$  of dissolved manganese and  $60 \text{ p.p.m. (w/v)}$  suspended solids.

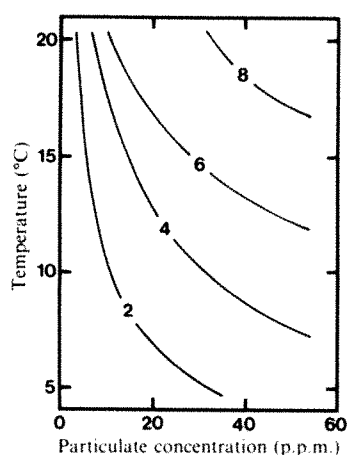


Fig. 2 Rate of zero-order loss ( $\mu\text{g l}^{-1} \text{d}^{-1}$ ) of dissolved manganese from solution in Tamar River water as a function of temperature and particulate load.

Temporal variations in the rate of manganese removal per mass of suspended particles are probably caused by an inherent natural variability in the surface characteristics of the particulates. The non-linearity of the relationship with respect to particulate concentrations at any one time is probably induced by increased aggregation of particles at higher concentrations of particulate material.

Although occurring within freshwater medium, the very rapid transfer of dissolved manganese to the particulate phase is an estuarine-induced phenomenon, in that the necessarily high concentrations of particles in the water column are generated and maintained by estuarine physical processes<sup>16</sup>. In the Tamar Estuary, cyclic spring-tide to neap-tide variability in the average amount of particulates comprising the turbidity maximum has been found to impart a corresponding variability in the rate and extent of removal of dissolved manganese in the freshwater immediately above the salt wedge. Furthermore, during neap-tide to spring-tide periods of increasing tidal amplitude, with pronounced net mobilisation of sediment, the removal of dissolved manganese may be more than counterbalanced by the infusion of sediment pore water containing relatively high concentrations of divalent manganese. Consequently the concentration of dissolved manganese in the freshwater entering the estuarine mixing sequence undergoes cyclic oscillation. This provides an alternative and/or complementary explanation for the common occurrence of a dissolved manganese maximum within estuarine water<sup>2-4,17,18</sup>. These maxima are usually attributed to desorption of manganese from riverine particles or to advective and diffusive influxes from the estuarine sediment although such explanations do not always seem to be quantitatively adequate<sup>2</sup>.

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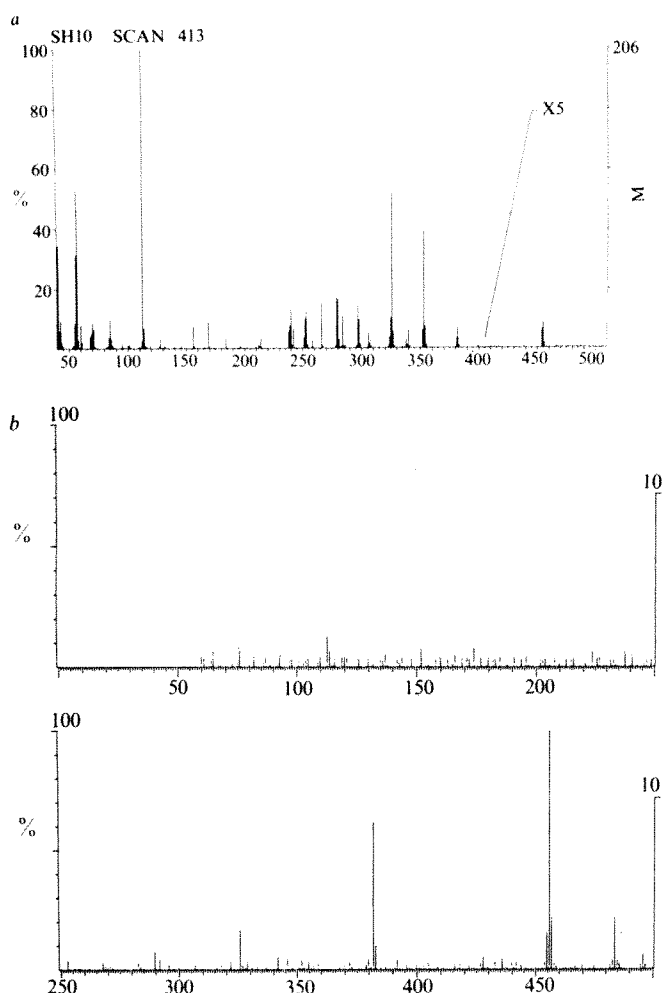
## Amino acids in interstitial waters of marine sediments

DISEQUILIBRIA within the mixture of organic matter, mineral particles and seawater in near-surface sediments result in extensive reaction, often biologically mediated. Variations in interstitial water composition are very sensitive indicators of the chemical and biological reactions in this zone of transition and the interaction between the water column and the permanent deposit<sup>1,2</sup>. Although most studies have been confined to inorganic ions, the few measurements of pore water DOC in marine sediments<sup>3-6</sup> show large gradients in dissolved organic matter concentration within sediments and across the sediment-water interface. We describe here our study of dissolved amino acids in interstitial waters, which were used to trace some of the biogeochemical processes affecting organic matter in marine sediments. We have analysed the dissolved free amino acids in 15 interstitial water samples from four cores obtained in Buzzards Bay, Massachusetts; the Gulf of Maine; and the North-west Atlantic continental rise. These pore waters have very high amino acid concentrations, of the order of  $1 \text{ mg l}^{-1}$ . In addition, the distribution of individual amino acids differs substantially from that reported for seawater<sup>7,8</sup>, particularly in the large relative abundance of glutamic acid and  $\beta$ -aminoglutaric acid.  $\beta$ -aminoglutaric acid ( $\text{HOOCCH}_2\text{CH}(\text{NH}_2)\text{CH}_2\text{COOH}$ ) is an isomer of glutamic acid which, to our knowledge, has not been previously reported in the marine environment.

Sampling was carried out using a 20 cm diameter sphincter corer<sup>9</sup> in Buzzards Bay and a 0.25 m<sup>2</sup> box corer (Sandia-Hessler Type MK III) in the Gulf of Maine and the North-west Atlantic. The Buzzards Bay (station P) sediment sampled was located in 17 m of water and was anoxic below the bioturbated upper 1-3 cm. Station 3 and station 8 in the Gulf of Maine were located at 250 m depth in the Wilkinson Basin and at 390 m in the Georges Basin, respectively. A small amount of  $\text{H}_2\text{S}$  was present in the deepest (24-28 cm) section at station 3, but none was detected in the 10 cm core from station 8. Station 10 was located on the continental rise east of the Gulf of Maine at a depth of 4,200 m. The sediment sampled was a strongly oxidising, foraminiferal ooze. Cores were sectioned immediately and the sediment subsamples refrigerated until squeezing was completed (within 24 h). Pore water was extracted from 100 to 200 g sediment using a hydraulically-powered stainless steel squeezer with two internal precombusted Reeve Angel<sup>(R)</sup> glass fibre filters and then refiltered through Gelman Type A<sup>(R)</sup> glass fibre filters (nominal particle diameter retained 0.3-1  $\mu\text{m}$ ). The filtered water and samples of whole sediment from corresponding core sections were frozen for onshore analysis.

After returning to Woods Hole, 10-20 ml pore water aliquots were applied to a 15 cm<sup>3</sup> Bio-Rad<sup>(R)</sup> AG 50W-X8 resin cation exchange column ( $\text{H}^+$  form) for desalting. The column was eluted with 25 ml water and then 1.5 M  $\text{NH}_4\text{OH}$  until the eluate began to become basic. The first 60 ml of the basic eluate containing the amino acids were collected, evaporated to dry-





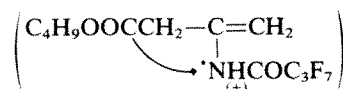
**Fig. 1** *a*, Electron-impact ionisation mass spectrum of  $\beta$ -aminoglutaric acid from station 3, 0–2 cm core section interstitial water; SE-52 glass capillary GC column interfaced with a Finnigan GC-MS 3200, ionisation voltage 70 eV. *b*, Chemical ionisation mass spectrum of  $\beta$ -aminoglutaric acid; SE-52 glass capillary GC column interfaced with a Finnigan GC-MS 1015 SL,  $\text{CH}_4$  reactant gas, ionisation voltage 130 eV.

ness, and the residue derivatised to form the *N*-heptafluorobutyl *n*-butyl esters<sup>10</sup> for gas chromatographic (GC) analysis. Blanks of the entire analytical procedure, including rinses of the squeezer and filters, give negligible concentrations of amino acids.

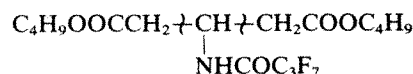
Gas chromatographic analyses were carried out on a 32 m  $\times$  0.3 mm i.d. SE-54 glass capillary column. The column was installed in a HP 5840 gas chromatograph with splitless injection at a column temperature of 40 °C and an injector temperature of 225 °C. After four minutes, the temperature was increased at 30 °C min<sup>-1</sup> to 70 °C, and then at 3 °C min<sup>-1</sup> to 240 °C. The carrier gas flow rate was 2–3 ml He min<sup>-1</sup> and the FID temperature was 250 °C. Derivatives of cysteine, histidine, and tryptophan were not detected in these conditions, and asparagine and glutamine were measured as aspartic and glutamic acids, respectively. Selected samples were subjected to gas chromatography–mass spectrometry (GC-MS) (Finnigan GC-MS 3200 or Finnigan GC-MS 1015 SL). Compounds were identified by comparison to GC retention times and mass spectra of amino acid standards (Sigma) obtained in the same conditions and with the same instruments used for samples.

$\beta$ -Aminoglutaric acid (which may occur to some extent as the glutamine-isomer amide) is a major amino acid in our pore water samples, making up 2–50% of the dissolved free amino acids. The chemical ionisation (CI- $\text{CH}_4$ ) and electron-impact ionisation (EI) mass spectra of the interstitial water amino acid (as the *N*-heptafluorobutyl *n*-butyl ester) which we have identified as  $\beta$ -aminoglutaric acid are

given in Fig. 1. The molecular ion at  $m/e$  455 (confirmed by the presence of  $M+1=456$ ,  $M+29=484$ , and  $M+41=496$  in the CI- $\text{CH}_4$  spectrum) indicates that the compound is an isomer of glutamic acid. The greater intensity of  $m/e$  353 ( $=M-102$ ) relative to  $m/e$  354 is characteristic of a  $\beta$ -amino acid rather than an  $\alpha$ -amino acid<sup>11</sup>. The  $m/e$  326 ion (probable structure  $\text{C}_4\text{H}_9\text{OOCCH}=\text{NHCOC}_3\text{F}_7$ ) may arise subsequent to a rearrangement of the  $m/e$  353 ion

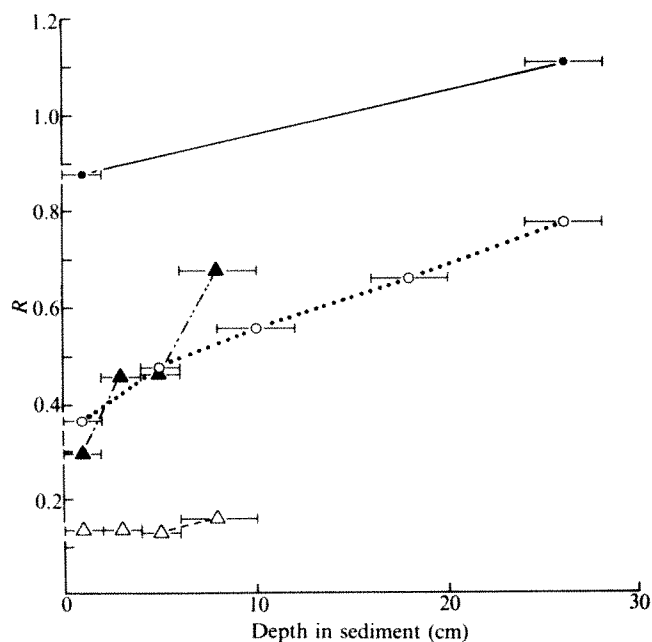


The base peak at  $m/e$  113 probably results from cleavage  $\beta$  to the amide N with loss of hydrogen:



The EI and CI- $\text{CH}_4$  mass spectra and glass capillary GC retention time of authentic  $\beta$ -aminoglutaric<sup>12</sup> acid are the same as those of the unknown compound in our samples. Those of the next most likely isomers of glutamic acid,  $\alpha$ -methylaspartic and  $\beta$ -methylaspartic acids, are markedly different.

Dissolved free amino acid concentrations in interstitial water are given in Table 1. Total concentrations in the samples analysed, ranging from 0.82 to 5.6 mg l<sup>-1</sup>, are two orders of magnitude greater than those found in nearshore waters, 5–30  $\mu\text{g l}^{-1}$  (ref. 7) or in open ocean seawater, 0–4  $\mu\text{g l}^{-1}$  (ref. 8). Concentrations generally reach a maximum within the upper 6 cm and then decrease with depth. Glutamic and  $\beta$ -aminoglutaric acids make up more than 90% of the dissolved free amino acids in station P interstitial waters, and from 76 to 90%



**Fig. 2**  $R$  ( $\beta$ -aminoglutaric acid/glutamic acid ratio): variation with depth in sediment and with overlying water column depth. ●, Station P (17 m); ○, station 3 (250 m); ▲, station 8 (390 m); △, station 10 (4,205 m).

of the total at station 3. Station 8 pore waters have significantly greater concentrations of other amino acids, especially alanine, glycine and serine, relative to glutamic and  $\beta$ -aminoglutaric acids which make up 40–75% of the total. Station 10 has a more uniform distribution of the protein amino acids and lower relative abundances of glutamic and  $\beta$ -aminoglutaric acids (18–35%).

Surface sediments from stations 3, 8, and 10 were hydrolysed in 6M HCl and the amino acids were analysed as described above. Concentrations range from 1.3 to 5.6 mg per g dry weight (Table 1), decreasing with overlying water column depth. The

**Table 1** Amino acid concentrations in interstitial waters\* and sediments†‡

	Ala	Gly	Val§	Thr	Ser	Leu	Ile	Pro	Asp	Phe	Glu	βGlu	Lys	Tyr¶	Total
<b>Interstitial water</b>															
<b>Station P</b>															
0–2 cm	36	19	— <sup>†</sup>	—	23	13	—	—	37	—	820	720	—	—	1.7
24–28 cm	24	57	—	—	—	—	—	—	70	—	620	680	—	—	1.4
<b>Station 3</b>															
0–2 cm	66	140	28	18	48	17	13	23	110	15	1,100	400	—	14	2.0
4–6 cm	59	42	39	28	40	33	19	10	81	20	1,800	860	—	—	3.1
8–12 cm	52	71	102	81	67	—	—	—	83	—	1,200	670	—	—	2.4
16–20 cm	28	28	14	32	49	—	—	—	40	—	630	410	—	—	1.2
24–28 cm	16	13	—	—	23	—	—	—	29	—	420	320	—	—	0.82
<b>Station 8</b>															
0–2 cm	360	1,500	360	85	110	120	82	110	260	75	1,600	480	—	81	5.2
2–4 cm	190	340	46	96	340	38	34	34	150	23	1,700	760	—	40	3.8
4–6 cm	400	990	73	44	110	81	57	30	94	33	1,300	560	—	34	3.8
6–10 cm	58	110	27	25	93	21	—	—	44	—	660	440	—	—	1.5
<b>Station 10</b>															
0–2 cm	490	480	320	190	210	240	220	50	420	60	940	130	—	18	3.7
2–4 cm	900	170	170	200	110	210	160	16	190	66	610	90	—	19	2.9
4–6 cm	1,500	780	360	290	210	480	360	57	330	170	880	110	—	97	5.6
6–10 cm	130	310	67	35	49	64	55	17	74	31	390	60	—	16	1.3
<b>Sediment hydrolysate</b>															
<b>Station 3</b>															
0–2 cm	0.63	0.83	0.49	0.42	0.43	0.32	0.20	0.26	0.89	0.22	0.54	—	0.23	0.06	5.6
<b>Station 8</b>															
0–2 cm	0.19	0.20	0.15	0.15	0.16	0.12	0.073	0.069	0.24	0.069	0.12	—	0.039	0.017	1.6
<b>Station 10</b>															
0–2 cm	0.14	0.18	0.077	0.10	0.11	0.078	0.043	0.056	0.16	0.047	0.098	—	—	—	1.3

\* Interstitial water dissolved free amino acid concentrations in  $\mu\text{g l}^{-1}$ , with depth-interval totals in  $\text{mg l}^{-1}$ .

† Sediment hydrolysate concentrations in mg per g dry weight.

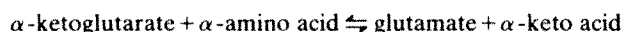
‡ Amino acid abbreviations are standard, except  $\beta\text{Glu} = \beta\text{-aminoglutaric acid}$ .§ Peak sometimes had shoulder or partially resolved component which may be  $\beta\text{-alanine}$ .

¶ Peak sometimes had shoulder or partially resolved component, probably due to small amounts of lysine.

¶  $<10 \mu\text{g l}^{-1}$  for interstitial water and  $<0.01 \text{ mg per g dry weight}$  for sediment hydrolysate. Some minor components, including hydroxyproline,  $\gamma\text{-aminobutyric acid}$ , ornithine and  $\alpha\text{-aminoadipic acid}$ , were omitted from this table.

amino acid compositions of the sediment hydrolysates are similar to those found by others for organic-rich near-shore sediments<sup>13–15</sup>.  $\beta\text{-Aminoglutaric acid}$  is present only in trace amounts ( $<0.4\%$  of the total hydrolysable amino acids), and glutamic acid makes up  $<10\%$  of the total. Thus, if pore water amino acids are supplied from detrital proteins in sediments, for example by bacterial proteolysis, either the process is selective for particular residues or proteins of very unusual composition, or the freed amino acids are further metabolised to produce the observed distribution.

The large relative abundance of glutamic acid in interstitial water may result from the following transamination which many organisms, including bacteria, carry out as a first step in the metabolism of some amino acids<sup>16,17</sup>:



Another potential source for dissolved free amino acids in interstitial water is excretion or loss of cellular fluids by higher benthic organisms. The magnitude and even the direction of the net flux of amino acids to and from benthic organisms such as polychaetes is the subject of some controversy<sup>18,19</sup>, and there is little information on the composition of these fluxes. Thus the significance of this source of amino acids cannot yet be evaluated.

The large relative abundance of  $\beta\text{-aminoglutaric acid}$  in pore waters is surprising, because we have been unable to find any reference to it as a natural product in the literature. In the 15 samples we have analysed so far, the  $\beta\text{-aminoglutaric acid}$  concentration is correlated ( $r = 0.81$ ) with the concentration of glutamic acid. In contrast, glycine, another abundant amino acid in some of our samples, shows no significant correlation. However, the levels of  $\beta\text{-aminoglutaric acid}$  do not depend solely on those of glutamic acid, as can be seen from Fig. 2, which plots the  $\beta\text{-aminoglutaric/glutamic acid ratio}$  ( $R$ ) in pore water against depth in core.  $R$  increases with depth in all four cores. Furthermore,  $R$  is non-zero in surface sediments, and this surface ratio decreases with increasing water depth from station P through stations 3 and 8 to station 10.

The concentration correlation between glutamic acid and  $\beta\text{-aminoglutaric acid}$  in pore water and the increase of  $R$  with depth in sediment suggests that  $\beta\text{-aminoglutaric acid}$  is being formed from glutamic acid. The fact that substantial amounts of  $\beta\text{-aminoglutaric acid}$  are present in surface sediments indicates that the reaction proceeds very rapidly and is probably biologically mediated. However, we have found no indication that such an  $\alpha \rightarrow \beta$  isomerisation is carried out by bacteria or other organisms except during lysine fermentation by certain *Clostridia*<sup>17</sup>. On the other hand, the inputs of  $\beta\text{-aminoglutaric acid}$  and glutamic acid may be by separate, but related processes. The increase in  $R$  with depth in core might then be explained by preferential metabolism or reduced inputs of glutamic acid rather than its conversion to  $\beta\text{-aminoglutaric acid}$ . In either case the decrease in the surface  $R$  from the shallow to the deep-water sediments is probably related to some characteristic(s) of the sedimentary environments, for example, redox potential and the related nature of the benthic community metabolising organic matter. We are currently analysing additional samples from a wider range of sediment types to improve our understanding of the relationship between pore water amino acid concentration and composition and sedimentary environment.

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## Reduced thermogenesis in obesity

It is often claimed that there are obese patients who find it difficult to maintain a normal body weight because they have such low energy requirements that even normal intakes of energy result in weight gain and obesity. Studies of both children<sup>1</sup> and adults<sup>2</sup> show that there can be a twofold difference in energy intake between individuals despite apparently similar patterns of physical activity. An individual variability in the capacity to dissipate heat by metabolic changes has therefore been suggested<sup>3</sup> but no physiological basis for the differences in thermogenesis has yet been established. In genetically obese *ob/ob* mouse there are two components involved in the deposition of excess body fat: hyperphagia and increased metabolic efficiency<sup>4,5</sup>. Metabolic efficiency is the major factor responsible for obesity when the animals are kept at 20 °C so these animals provide a model study of the link between metabolic rate and obesity. Pre-obese and obese *ob/ob* animals have an abnormality of thermoregulatory thermogenesis with a reduced thermogenic response to cooling<sup>6</sup>. A defect in non-shivering thermogenesis can be confirmed by monitoring the thermogenic response to maximum doses of noradrenaline: the *ob/ob* mouse has only half the response of its lean littermate. The abnormal thermoregulatory thermogenesis quantitatively accounts for most of the metabolic efficiency of the obese animals as pair feeding at thermoneutrality rather than at 23 °C reduces the excess fat deposited by 65%<sup>7</sup>. We report here that obese adults with a family history of obesity have a reduced metabolic response to noradrenaline infusion compared with thin adults. As the reduced non-shivering thermogenesis is also found in subjects with familial obesity who remain at normal weight by persistent dieting, the defect in non-shivering thermogenesis appears to be constitutional and not a secondary consequence of obesity.

Thermogenic responses were tested in six obese women, aged  $47 \pm 4.5$  yr (mean  $\pm$  s.e.m.), who claimed to gain weight readily and who had a strong family history of obesity. A group of seven lean women of equivalent age ( $49.5 \pm 2$  yr), with no family history of obesity, served as controls. All seven claimed that they were able to eat freely and had no problems with weight gain. A third group of 'post-obese' women (aged  $44.2 \pm 5.4$  yr) was also studied once they had slimmed from an obese state after 1–2 yr on a severely restricted diet. They had maintained a stable weight by careful dieting for at least 3 months before the test. The subjects in all three groups were euthyroid and normotensive.

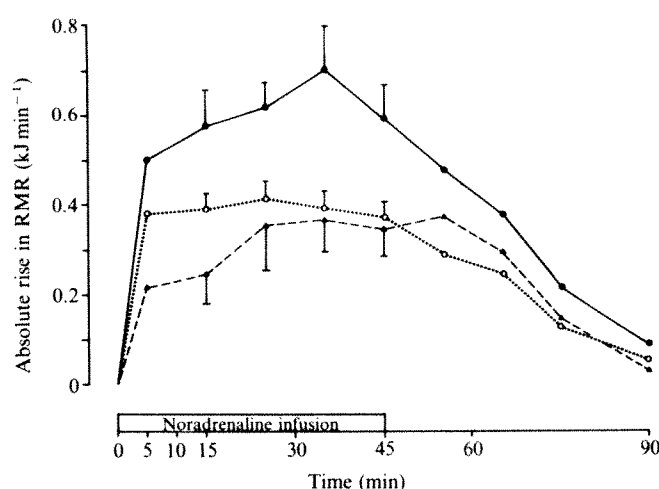
The metabolic capacity for thermogenesis was tested by infusing noradrenaline intravenously in a dose related to the ideal body weight (IBW). Tests were conducted at the same time

of year with all subjects in a weight-stable condition. Subjects were fasted overnight for 12 h and were tested in identical clothing in thermoneutral conditions (27.2–27.6 °C) after allowing 1 h for heat equilibration.

The resting metabolic rate (RMR) was measured at 1-min intervals for 30 min after the subject had rested supine for 30 min. Noradrenaline (Levophed, Winthrop) was then infused intravenously ( $0.1 \mu\text{g}$  per kg IBW per min) through a brachial vein for 45 min. The intravenous dose of noradrenaline was chosen to produce in the other arm a venous plasma noradrenaline corresponding to that found in moderately severe exercise. An indwelling Abbott 19-gauge venous cannula, kept patent with 3.8% sodium citrate (BPC) and inserted in the non-infused arm, was used to remove blood samples for assay. The RMR was measured continuously during the infusion and for 45 min after the infusion had stopped.

Immediately on starting the infusion of noradrenaline, the RMR increased and remained at near plateau levels for the duration of the infusion (Fig. 1). The rise in the RMR in the lean group was striking, amounting to an increase of 21.2%. In both the obese and post-obese subjects, however, the rise in RMR was only half that seen in the lean subjects, that is, 9.6% (Table 1). This difference in thermogenesis was seen despite the fact that similar rises in plasma noradrenaline were achieved in the three groups. Although the RMR in the lean group during the infusion was still less than that in the obese patients, similar metabolic rates were found during infusion in the lean and post-obese groups. The high pre-infusion RMR of the obese group was consistent with similar measurements made on other days and was therefore not due to stress but was related to their increased lean body mass<sup>8</sup>. The plasma adrenaline levels remained unchanged during the basal period and were similar in all three groups. There was also no increase in plasma adrenaline during the infusion, again indicating that stress played no part in the thermogenic differences.

During the infusion there was no significant difference between the groups in the rise of either the free fatty acids (FFA) or glucose (Table 1). However, the obese group showed a greater rise in the plasma glycerol and a marked increase in the insulin/glucose ratio, indicative of insulin resistance. As changes in plasma glycerol are considered a better index of changes in lipolysis than FFA responses, these results suggest that the lipolytic response to noradrenaline was greater in the obese. The



**Fig. 1** Absolute increase in RMR of women during and after a 45 min intravenous infusion of noradrenaline. ●, Lean; ○, obese; ▲, previously obese, now lean. RMR was measured by the ventilated hood technique using a Servo paramagnetic oxygen analyser and IR carbon dioxide analysis<sup>20</sup>. RMR measured by this technique agrees with direct calorimetry with a difference of 0.09%<sup>8</sup>. Values are means  $\pm$  s.e.m. Fully informed consent for the procedures was obtained and ethical approval was given by the unit's ethical committee.



**Table 1** Basal and peak hormone and substrate concentrations during noradrenaline infusion

Subjects	Weight (kg)	Mean% of ideal body weight <sup>17</sup>	Noradrenaline (ng ml <sup>-1</sup> )		Free fatty acid (μmol l <sup>-1</sup> )		Glycerol (μmol l <sup>-1</sup> )		Resting metabolic rate (kJ min <sup>-1</sup> )		Insulin to glucose ratio	
			Basal	Absolute rise	Basal	Absolute rise	Basal	Absolute rise	Basal	Absolute rise	Basal	At peak† insulin response
Lean (7)	49.5 ± 2.0	-11	0.298 ± 0.052	1.015 ± 0.222	622 ± 31	796 ± 79	57.4 ± 3.9	89.6 ± 14.6	3.326 ± 0.102	0.705 ± 0.104	2.05 ± 0.18	2.90 ± 0.39
Obese (6)	84.1 ± 5.6	+53.3	0.281 ± 0.057	1.225 ± 0.178	712 ± 100	962 ± 56	78.5* ± 7.9	152.7** ± 16.8	4.309*** ± 0.207	0.415* ± 0.042	3.20* ± 0.48	4.30* ± 0.37
Post-obese (6)	65.3 ± 2.7	+15.6	0.230 ± 0.055	0.812 ± 0.149	579 ± 81	947 ± 97	80.3 ± 11.0	135.8 ± 21.7	3.849* ± 0.174	0.371* ± 0.065	2.15 ± 0.37	2.76 ± 0.48

Number of subjects given in parenthesis. Values are means ± s.e.m. Insulin to glucose ratio = insulin IU l<sup>-1</sup>/glucose mmol l<sup>-1</sup>. Significance values represent comparison of obese or post-obese with lean.

\* =  $P < 0.05$ ; \*\* =  $P < 0.02$ ; \*\*\*  $P < 0.01$ .

† Glucose rises during infusion and falls once infusion is stopped. However, insulin release is initially inhibited by the noradrenaline infusion but rises once the infusion is stopped—hence the ratio is measured again at the peak of the insulin response about 15 min after the infusion is stopped. Noradrenaline is measured by radioenzymatic assay<sup>18</sup>, FFA by titration<sup>19</sup>, glucose using a Beckman Autoanalyser, glycerol by an enzymatic assay system (Boehringer Mannheim no. 125032) and insulin by radioimmunoassay (Amersham kit no. 1M78).

defective heat production in the obese therefore cannot be explained by a subnormal rate of lipolysis. The equivalent FFA concentrations in all three groups also suggest that substrate availability for energy metabolism was not a factor in the different rates of thermogenesis. Issekutz *et al.*<sup>9</sup> have shown that the oxidation of FFA depends on the plasma concentration and that the relationship between plasma concentration and the rate of oxidation was the same in lean and obese subjects. A thermogenic system directly responsive to catecholamines therefore seems to be involved. The noradrenaline concentrations at the site of thermogenesis cannot be ascertained from this study but the markedly increased and equivalent venous noradrenaline levels in the three groups during the infusion and the similar metabolic response to noradrenaline found in this study compared with that observed previously in cold-adapted man<sup>10</sup> infused with noradrenaline at 150% of the rate used in our study, suggest that we were close to if not at the limits of the non-shivering thermogenic capacity of all three groups.

The difference between the lean and obese groups in the thermogenic action of noradrenaline is strikingly similar to that seen in genetically obese rodents with their defects in thermoregulatory thermogenesis.<sup>6</sup> In adult rats it is now evident that the process of non-shivering thermogenesis is dependent on the continued capacity of brown fat in the thoracic, cervical and subscapular areas to generate heat by mechanisms responsive to noradrenaline<sup>11</sup>. The metabolic activity of brown fat when stimulated is far in excess of that expected on a weight basis. Abnormalities of mitochondrial function have recently been reported in the brown fat of *ob/ob* mice<sup>12</sup>, but these abnormalities may be secondary to changes in the hypothalamus<sup>13</sup> as the thermoregulatory system of these animals is controlled so that body temperature is maintained approximately 2 °C below their lean littermates throughout the diurnal temperature cycle and at environmental temperatures varying from 10 to 25 °C (ref. 5). Below 10 °C, however, the *ob/ob* mouse becomes very hypothermic.

Our studies suggest that a similar mechanism may be involved in adult human subjects, as brown fat has been identified even in elderly men and women<sup>14</sup>. The defective thermogenic response of the obese women conforms with the reports of their enhanced susceptibility to body cooling<sup>15,16</sup> despite the additional insulation of subcutaneous fat. The defect in the women seems to be unrelated to energy intake, as two obese women who were deliberately fed 40 kcal per kg IBW per day for 7 days before testing responded to noradrenaline in a similar manner to that

after 3 weeks on a diet providing 9.2 kcal per kg IBW per day. Although an adaptive increase in the metabolic response to noradrenaline in man has been reported, the effect was observed only after exposure to prolonged and severe cold<sup>10</sup>.

We suggest, therefore, that the thermogenic abnormality in the obese women is constitutive rather than the result either of developmental or other environmental factors. If a direct relationship can be established between the thermogenic defect and metabolic efficiency in human subjects as well as in animals, then this test could prove to be a useful method for assessing the propensity to develop obesity in man.

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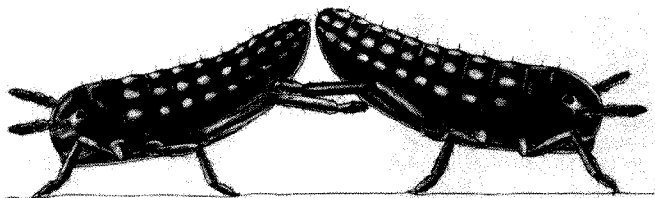
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## Territorial behaviour of *Pemphigus* gall aphids

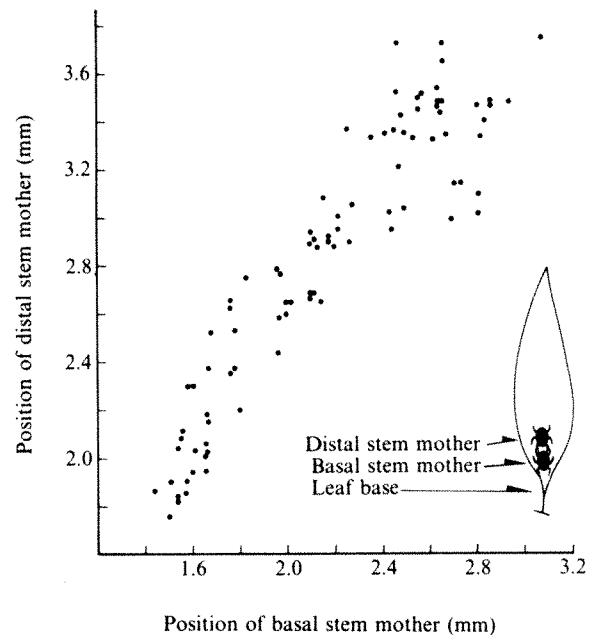
ALTHOUGH insects are known to defend nests, breeding sites and females<sup>1</sup>, the defence of feeding sites is less well documented. Other than the defence of egg clutches and nymphs by female treehoppers<sup>2</sup> and the existence of a soldier caste in woolly aphids<sup>3</sup>, territoriality has not been reported in the large insect order Homoptera which includes aphids, scale insects, hoppers, cicadas and whiteflies. As the evolution of territoriality is thought to be directly correlated with competition for resources in short supply<sup>4</sup>, territorial behaviour should only be exhibited when population densities approach the carrying capacity of the environment. Because parthenogenetic reproduction and the high population growth rates of aphids seem contradictory to the notion of limited resources, aphid territorial behaviour is not expected. I report here on the settling behaviour of the aphid, *Pemphigus betae* Doane, which forms galls of the leaf blade of narrowleaf cottonwood, *Populus angustifolia*. We have quantified the existence of a defended micro-territory, the production of a floater population of individuals displaced through competitive interactions, and the differential mortality of residents and floaters which favours the evolution of territorial behaviour.

Field observations and experiments were carried out in the springs of 1976 and 1978 near Ogden, Utah. As leaf buds begin to break in early spring, colonising stem mothers of *P. betae* emerge from overwintering eggs and migrate from the base of the tree to developing leaves. First instar stem mothers are black, about 0.6 mm long, and although wingless, are highly mobile. Within 4 or 5 days of arrival, most have settled and begun to methodically probe immature leaf tissues with their stylets to induce gall formation. Each stem mother is soon enclosed within a hollow gall where up to 300 progeny are produced parthenogenetically. Survival, number of progeny, and other measures of relative fitness are correlated with gall position on the leaf blade and mature leaf size<sup>5,6</sup>. When two or more stem mothers occupy the same leaf, they arrange themselves linearly along the midrib. Stem mothers occupying the base of large leaves achieve the greatest success, and these gall sites are highly preferred<sup>5,6</sup>.

When competitor densities exceed the number of superior gall sites available, territorial interactions occur during the brief colonisation phase before stem mothers become physically isolated by expanding leaf gall tissue. The outcome of these interactions is determined by kicking and shoving contests (Fig. 1) in which the largest stem mother usually wins or successfully defends a linear territory of about 3 mm at the base of the leaf blade. Body size was conservatively measured as the width of the semi-sclerotised prothorax. From microscope measurements of 54 pairs of first-instar competing stem mothers, the average prothorax width of basal stem mothers was 6.3% greater than that of their distal competitors (two-tailed paired *t* test,  $t = 3.298$ ,  $P < 0.005$ ). In agreement with these findings, Whitham<sup>6</sup> demonstrated that the removal of the defending basal stem mother can result in the downward shift of the remaining stem mother to occupy the superior basal position.



**Fig. 1** Typical back-to-back fighting posture of two competing stem mothers. The largest stem mother usually wins the superior basal position. Drawing by Pam Lungé.



**Fig. 2** Results of a 2-d contest in which the position of the subdominant distal stem mother is plotted as a function of the position of the dominant basal stem mother. Linear regression analysis ( $r = 0.934$ ,  $n = 88$ ,  $P < 0.001$ ,  $y = 1.17x + 0.28$ ) demonstrates that during contests, the position of each stem mother is based on the position of its competitor. Because stem mothers linearly arrange themselves along the midrib of the leaf blade, a single measure obtained with a caliper (the distance from the centre of each stem mother to a point marked at the base of the leaf) accurately quantifies position.

Contests can last 2 days and the basal stem mother can be displaced from her position. Figure 2 shows results from such a contest which began when a second stem mother arrived at an occupied leaf and displaced the resident to a suboptimal distal position. Positions were recorded every 10–15 min during daylight hours. During 44 of 88 recordings made, stem mothers were engaged in kicking and shoving contests in which no feeding or gall-forming activities occurred. The position of the subdominant distal stem mother on the leaf blade is plotted as a function of the position of the dominant basal stem mother. The high degree of correlation ( $r = 0.934$ ,  $P < 0.001$ ) indicates that stem mothers move like boxers in a ring, continually sparring to determine the winner. On the third day, only one stem mother remained. This example of an extended contest is unusually long for insects. Most observations of territorial contests have shown that the outcome is determined within a few minutes<sup>7–9</sup>. If contest time is evolutionarily determined, then contests lasting more than a few minutes suggest that great dividends or losses are at stake<sup>10</sup>.

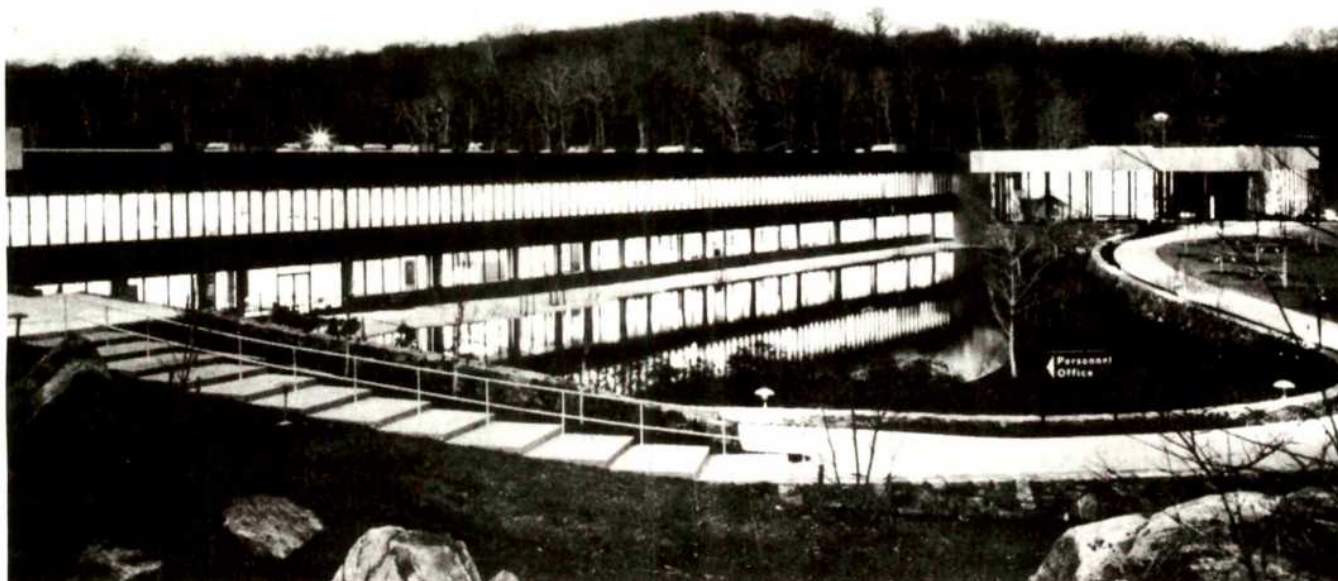
Territorial interactions can produce a floater population of stem mothers searching for places to settle. Two adjacent trees differing greatly in the densities of competing stem mothers were selected for removal experiments. On one arm of selected Y-shaped twigs on both trees, all arriving stem mothers were removed every day for a period of 7 days; the control arm of each twig was sampled only on day 7. It was expected that on the high density tree where competitive pressures for limiting gall sites should be greatest, individuals of inferior competitive abilities would accumulate on removal twigs due to competitive release. In this case, the sum of all stem mothers removed would be greater than the number of stem mothers on control twigs. In comparison, on the low density tree, competitive pressures should be much reduced, enabling stem mothers to settle quickly without further movement. Consequently, few or no differences were expected in the settling patterns of stem mothers on removal or control twigs. On each tree approximately 2,500 leaves were examined. Figure 3 shows the pattern of stem

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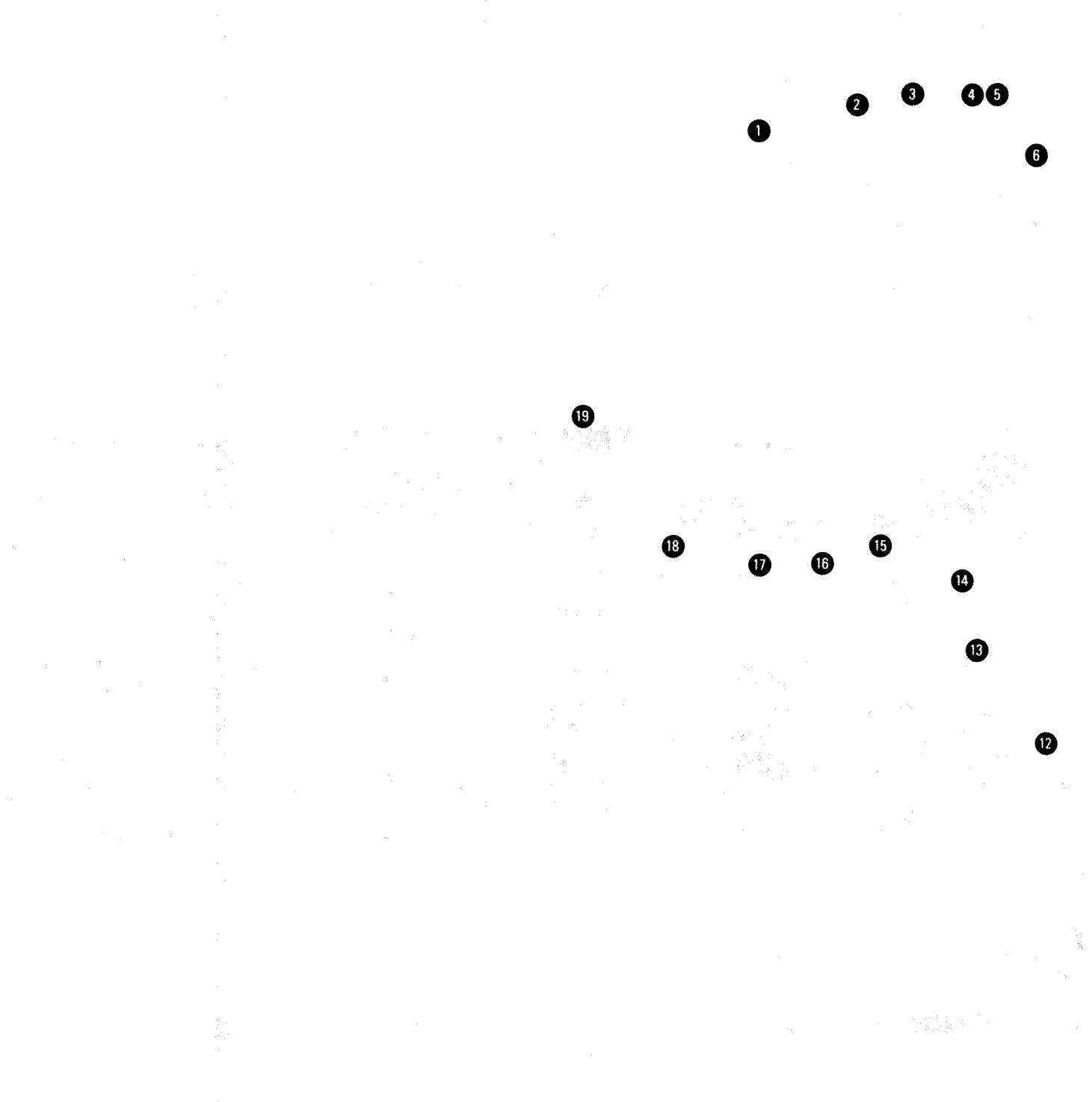
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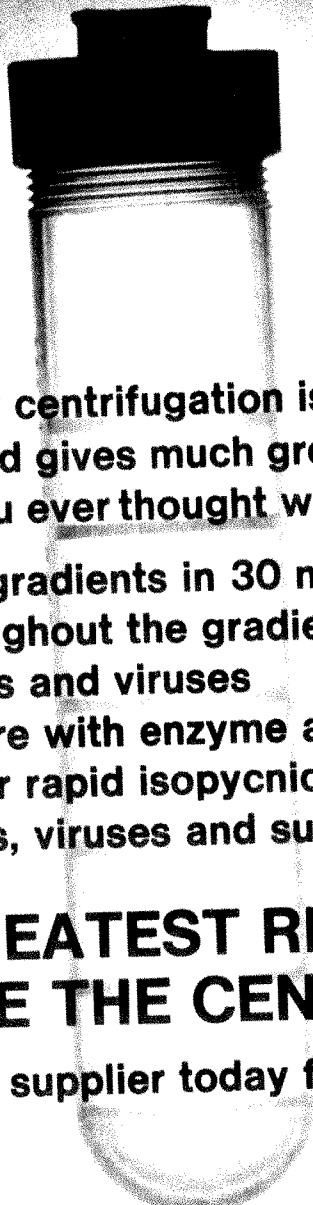
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mother arrival on removal twigs. Of the 699 stem mothers attempting colonisation on the high density tree, 83% arrived over a 3-d period that peaked just after bud burst; on the low density tree, colonisation spanned the same time period but the peak was much reduced. Figure 3b, shows that when competitor densities are high, colonising stem mothers rapidly respond to the removal of competitors. The total number of stem mothers removed during seven consecutive days of sampling (535 per 1,000 leaves) was 69% greater than the number of stem mothers found on control leaves (316 per 1,000 leaves) ( $\chi^2 = 46.67$ ,  $P < 0.001$ ). On the tree with a low competitor density, however, the number of stem mothers collected on removal twigs (72 per 1,000 leaves) did not differ significantly from the number of stem mothers found on control leaves (61 per 1,000 leaves) ( $\chi^2 = 1.11$ ,  $P > 0.29$ ). The fact that there was a highly significant difference between treatment and control on the high density tree shows that stem mothers were moving in search of a place to settle and were reacting negatively to the presence of other stem mothers. On the other tree, however, densities of potential competitors were so low that competition for a limited number of gall sites was reduced, enabling all stem mothers to rapidly select leaves and immediately settle. Thus, with high population densities, many stem mothers are displaced through competitive interactions and a floater population is produced. These results are similar to those obtained in other systems as diverse as birds, mammals and fish, where subdominants or floaters moved into vacated spaces<sup>11-17</sup>.

Experiments indicate that the floater population suffers much greater mortality than the resident population. Before all stem mothers had permanently settled during colonisation, the positions of 211 stem mothers distributed among 351 leaves of a small branch were recorded. A sticky barrier (Tanglefoot) was placed at the base of the branch to prevent further recruitment. After all stem mothers had permanently settled, their positions were again recorded. Forty stem mothers had abandoned the positions they had held during the first census and attempted gall formation on other leaves. Of these individuals, only 24% survived, while 72% of those that did not move from their original positions survived ( $\chi^2 = 21.45$ ,  $P < 0.001$ ). This experiment has been repeated with another *Pemphigus* species and the results were identical<sup>5</sup>. Such differential mortality could be an important factor in the evolution of territorial behaviour and may account for the observed extended contest of Fig. 2.

The Eriosomatidae, or gall-making aphids of which *Pemphigus* is a member, exhibit a unique reproductive trait in which each stem mother is the sole progeny of a female sexual<sup>18</sup>. As

no other aphid group or known animal lays only one egg throughout their lifespan, this trait must have evolved under rather severe and/or unusual circumstances. I suggest that the advantage large body size confers in territorial interactions may have contributed to the evolution of this trait by favouring the reduction of clutch size and the placement of all resources into a single large egg.

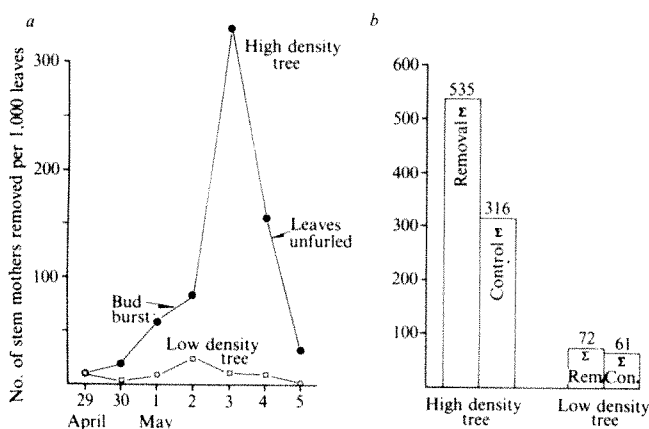
I thank J. Alcock, H. R. Pulliam, C. C. Smith and W. V. Zucker for comments on the manuscript and acknowledge the support of NSF grant no. DEB 7905848.

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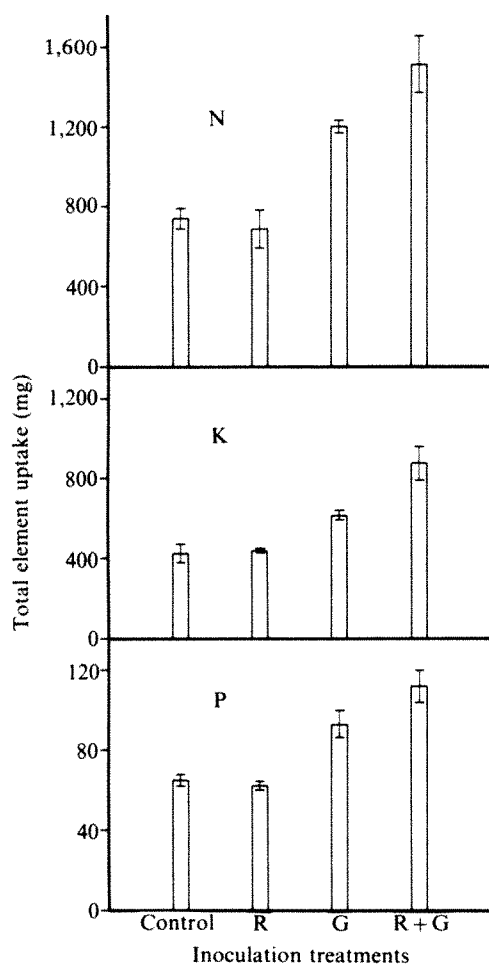


**Fig. 3** Results of a removal experiment designed to test the hypothesis that due to territorial interactions occurring during leaf selection, a floater population is produced. Y-shaped twigs on two trees which differed in the density of competing stem mothers were selected. One arm of each twig acted as the control and was sampled only at the end of the experiment. On the other arm stem mothers were removed each day. *a*, Shows the number of stem mothers removed each day from both trees; *b*, compares the removal and control twigs for both trees (see text).

## Endomycorrhizal fungi and *Rhizobium* as biological fertilisers for *Medicago sativa* in normal cultivation

LEGUMES can form two types of symbiotic association with microorganisms. One, with *Rhizobium* sp., is involved in the fixation of atmospheric nitrogen; the other, with fungi of the family Endogonaceae that form vesicular-arbuscular (VA) endomycorrhizas, is concerned with the uptake of phosphorus by the plants. Glasshouse experiments have demonstrated that legumes inoculated with both types of microorganism grow and nodulate better, and have higher nitrogenase activity and phosphorus content than plants that are uninoculated or inoculated with either *Rhizobium* or mycorrhizal fungi separately<sup>1-12</sup>. Also, plants with both types of symbiosis may be important as pioneer colonisers of nutrient-deficient habitats<sup>13</sup>. At present, the possibility of field inoculation with mycorrhizas to improve yield, and the subsequent economy in the use of chemical fertilisers, are being considered<sup>14</sup>. Positive responses to VA mycorrhizas are to be expected mainly in soils low in nutrients, particularly phosphate, and where indigenous endophytes are sparse or inefficient<sup>14</sup>. Thus, the use of soil sterilisants to destroy indigenous endophytes has been assayed<sup>15-16</sup>. There are also reports on the effect of VA fungi inoculation in non-sterile soils<sup>17-20</sup>. The effects of endomycorrhizas on legumes in relation to the improvement of hill<sup>19-20</sup> and marginal soils are now being studied<sup>21</sup>. But as far as we know no data for leguminous crops growing on non-sterile arable soils in standard agricultural conditions in temperate regions have been published. We report here that inoculation of *Rhizobium* and *Glomus* improves the growth and nutrition of *Medicago sativa* in normal cultivation on an arable field.

The experiment was carried out on an irrigated calcareous soil (pH, 7.8) in a fertile valley ('vega') in Granada province, Spain. Its texture was: 25.2% sand, 30.0% loam and 44.8% clay. The soil contained 1,302 p.p.m. total N, 415 p.p.m. total K,



**Fig. 1** Effects of *Glomus* (G) and *Rhizobium* (R) on N, P and K uptake by *Medicago sativa* growing in an arable field soil under normal cultivation. Total N, P and K taken up by the plants is calculated from data of shoot dry weights and the percentage of element (mean of four replicates). Standard errors are shown.

611 p.p.m. total P, 9.2 p.p.m. available phosphate<sup>23</sup> and 1.74% organic matter. This field has been intensively cultivated for centuries and two crops per year are harvested. When the present experiment was designed the field had been left fallow for six months. Endogonaceae spores were recovered by the technique of wet sieving and decanting<sup>22</sup>. The number of spores per 100 g soil was:  $14 \pm 1.4$  yellow vacuolate<sup>24</sup> (*Glomus mosseae*<sup>25</sup>),  $2 \pm 0$  laminate<sup>24</sup> (*Glomus macrocarpus*<sup>25</sup>) and  $8 \pm 0.7$  unidentified. Spore numbers were, therefore, low but fall in the range of about 0.1–5 per g soil, recovered in most cases<sup>26</sup>. The predominance of *Glomus mosseae* spores in this field would justify, from the ecological point of view, the choice of this species as inoculant.

*Medicago sativa* L. cv. Aragón was the host plant for an endomycorrhizal fungus of the yellow-vacuolate spore type and for *Rhizobium meliloti* 203. Four seedbeds were prepared for plants for the field experiment. These were: uninoculated control (C), *Rhizobium*-inoculated (R), *Glomus*-inoculated (G) and *Rhizobium* + *Glomus* inoculated (R+G). The seedbeds were kept for 20 d in a glasshouse at 19–25°C. At this time, seedlings had a slight VA infection; about 3–5% of their root system. The experimental field was divided into four plots: C, R, G and R+G, to each of which seedlings from the corresponding seedbed were transplanted. Each plot consisted of four replicates with each replicate containing five 1 m<sup>2</sup> microplots. These microplots received 25 groups of three plants. Care was taken in selecting uniform seedlings and allowing some soil from the seedbed to adhere to their roots. Seedlings of R and R+G treatments were reinoculated with *Rhizobium* at transplanting.

**Table 1** Effects of *Glomus* and *Rhizobium* on the yield of *Medicago sativa* growing under normal cultivation in an arable field soil

Inoculation treatments	Shoot dry weight (g)
Uninoculated controls	15.18 ± 0.95
<i>Rhizobium</i>	15.94 ± 1.19
<i>Glomus</i>	22.56 ± 1.11
<i>Rhizobium</i> + <i>Glomus</i>	32.09 ± 1.77

Mean of four replicates.

Plants were irrigated by the farmers in their usual way, and no chemical fertilisers were applied during the experiment. At harvest, plants which grew during 12 weeks in the five microplots belonging to the same replicate were pooled, dry weights of shoots were recorded and analysed for P, N and K (ref. 27).

Table 1 records plant growth. It is clear that *Glomus* inoculation improved the yield of alfalfa, whereas *Rhizobium* inoculated alone did not. Uninoculated control plants showed nodulation by indigenous rhizobia, however, in *Glomus*-inoculated plants the introduction of *Rhizobium* was effective and plant dry weight was increased by nearly 50% (R+G treatment compared with G treatment). The available P content in the test soil is low; this probably not only determined the response of the plants to *Glomus* inoculation but also that plants not given a *Glomus* inoculum did not respond to *Rhizobium* inoculation. Phosphorus is known to be an essential element for N<sub>2</sub> fixation<sup>8,21</sup> and as it was a limiting factor in this experiment, plant growth and *Rhizobium* activity were not greater than for the uninoculated controls unless the plants were adequately mycorrhizal. *Glomus* inoculation improved total N, P and K uptake and the inoculation with *Rhizobium* was only effective when applied together with *Glomus* (Fig. 1).

The main conclusion from these results is that the mycorrhizal fungi *Glomus mosseae* is an effective 'biological fertiliser' for *Medicago sativa*. Inoculation with *Glomus* not only affected plant growth and nutrition but also enhanced the activity of *Rhizobium meliloti* when it was applied as inoculant. The introduction of *Rhizobium* together with *Glomus* and the subsequent establishment of effective dual symbiotic association with alfalfa was successful, as yield was increased by 211% compared with control. This suggests that it may be possible to reduce the chemical fertiliser inputs to this arable crop. This study may have practical significance for neutral-alkaline soils like the one used in the present investigation. As this soil is under intensive cultivation it has received, and is receiving, large amounts of organic and inorganic additives; as a result, the total P is high but the available P is quite low, probably because the added P fertiliser has become fixed, mostly by the calcium in this high pH soil, or by some minerals of the clay fraction<sup>28</sup>, which is relatively high in the test soil. The extensive use of fertilisers also accounts for the low number of Endogonaceae propagules<sup>29</sup>.

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## Anomalous temperature dependence of the sodium conductance in rabbit nerve compared with frog nerve

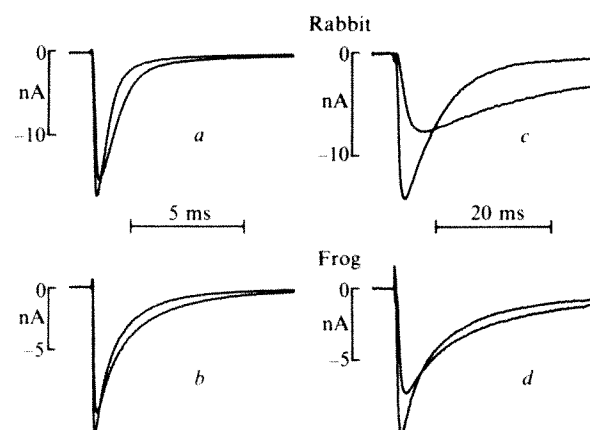
THE sodium channel in excitable tissues is an integral component of the membrane, and is intimately associated with the surrounding lipid<sup>1</sup>. Thus, the electrophysiological parameters of the sodium channel might be expected to exhibit a characteristic dependence on temperature, reflecting the marked temperature sensitivity of the physical properties of most lipids. We report here voltage-clamp experiments which show that cooling a rabbit node below the region of about 6 °C does in fact sharply decrease the maximal sodium conductance, and markedly prolongs sodium inactivation. It thus seems that the lipid (or lipid-protein) environment of the sodium channel in the rabbit node undergoes a drastic change below 6 °C.

Single myelinated fibres from rabbit and frog (*Rana pipiens*) sciatic nerves were dissected and voltage-clamped<sup>2</sup>. The nerve chamber was initially allowed to stabilise at room temperature (25 °C). Pre-cooled liquid was then pumped through the brass block enclosing the nerve chamber, gradually cooling the whole preparation to about 0 °C in 15 min. The temperature was measured throughout the experiment by a small thermocouple (50 µm diameter) located about 1 mm beneath the node in the pool containing the node. A single fixed depolarisation to –20 mV (rabbit) or to –5 mV (frog), preceded by a hyperpolarising prepulse to –125 mV to remove sodium inactivation, was used to assay sodium conductance every 0.3–1 °C as the temperature fell from 25 to 0 °C. At the end of the experiment, the temperature dependence of the internodal resistance was measured over the same temperature range and used to correct for consequent changes in current. Typically, the internodal resistance increased progressively, rising by a factor of about 1.3 in both frog and rabbit nerve when the temperature fell by 10 °C. The net ionic current associated with the test pulse in a rabbit node consisted of an inward current superimposed on an outward leak current, potassium current being virtually absent in the mammalian node<sup>2,3</sup>. In frog nerve, tetraethyl-ammonium was added to block potassium current. After subtracting the leak component from the total ionic current, the values of  $\tau_h$  for both frog and rabbit nodes were determined by fitting the sodium current to the expression:

$$I_{Na} = G_1 e^{-t/\tau_h} (1 - e^{-t/\tau_m})^p$$

with  $p = 2$  for rabbit and  $p = 3$  for frog.

Figure 1 shows typical sodium current records from a frog and rabbit node of Ranvier at different temperatures. A 5 °C fall in temperature from 23 °C slowed the kinetics of the sodium

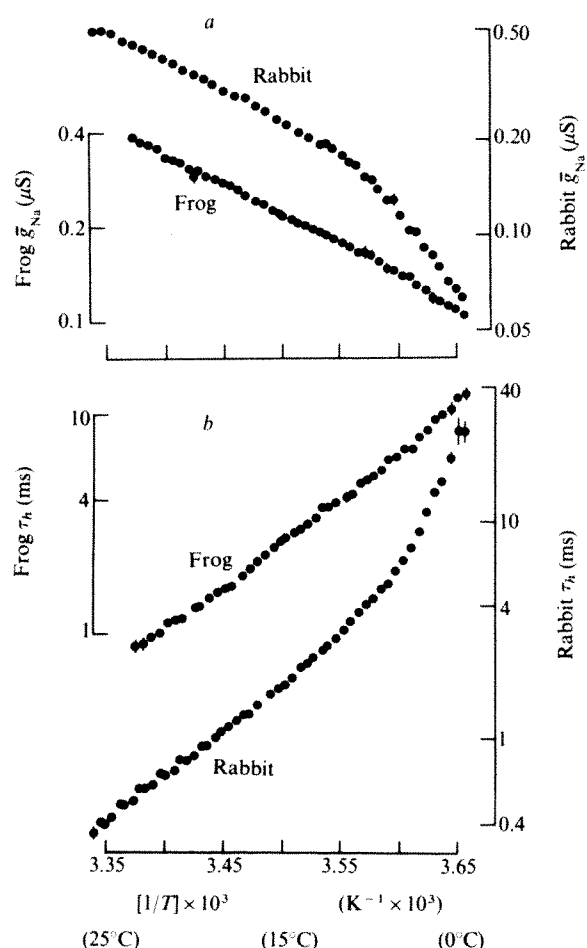


**Fig. 1** Sodium currents in a rabbit (a, c) and frog (b, d) node. Each pair of currents shows the response at a given temperature (23 or 6 °C) superimposed on one at a temperature 5 °C lower: a and b, 23–18 °C; c and d, 6–1 °C. In the rabbit  $E_{Na}$  was +70 mV at 25 °C and +65 mV at 0 °C; in the frog the corresponding values were +58 and +65 mV, respectively. The sodium current after leak subtraction and correction for temperature-dependent changes in the internodal resistance was calibrated by assuming a value of 10 mΩ for the internodal resistance at 25 °C. Note that the time to peak is increased relatively more in c than in a.

current in both frog and rabbit nerve by about the same extent and produced a similar decrease in the size of the current (Fig. 1a, b). However, a 5 °C fall in temperature from 6 °C produced a much bigger decrease in the peak current and a larger prolongation of sodium inactivation in the rabbit than in the frog (Fig. 1c, d). These effects of cooling were reversible on re-warming.

Figure 2 shows in greater detail the temperature dependence of the maximal sodium conductance,  $\bar{g}_{Na}$ , and the time constant of inactivation,  $\tau_h$ , for five rabbit and six frog nodes. The value of  $\bar{g}_{Na}$  was determined by extrapolating the decay phase of the sodium current back to the onset of the test pulse at each temperature. Figure 2 shows clearly that the curves for the temperature dependence of these parameters in frog and rabbit nodes have different shapes; in the frog the slope of the curve remains roughly constant over the whole temperature range<sup>4,5</sup>, whereas in the rabbit there is a marked change in slope at about 6 °C. Thus, the value of  $\tau_h$  for rabbit at –20 mV increased by a factor of about 1.73 for a 5 °C drop in temperature from above 15 °C ( $Q_{10}$  about 3), whereas below a transition temperature region (about 6 °C) the same drop in temperature increased  $\tau_h$  5.7-fold ( $Q_{10}$  about 33). Similarly, the values for  $\bar{g}_{Na}$ , which depended only moderately on temperature above 6 °C ( $Q_{10}$  about 1.7), became much more sensitive to temperature below 6 °C, the  $Q_{10}$  for the decrease being about 4.7. Because the rising phase of the sodium current was relatively fast, and because relatively few computer sampling points were taken during it, measurements of  $\tau_m$  were too uncertain for us to determine whether or not a similar discontinuity occurred with this parameter.

Several factors besides a reversible reduction of  $\bar{g}_{Na}$  could have caused the marked decrease of sodium current at low temperatures: for example, a reduction in  $E_{Na}$  or shifts of the  $h_{\infty}(E)$  and  $P_{Na}(E)$  curves with temperature. In the present study, the average values of  $E_{Na}$  at 25 °C and 0 °C were both 65 mV for frog nodes, and 65 mV and 60 mV, respectively, for rabbit nodes. A decrease of as much as 10 mV on cooling would have decreased the sodium current associated with the test pulse (at –20 or –5 mV) 1.2-fold at most. Furthermore, although the  $h_{\infty}(E)$  curves in both frog and rabbit were consistently shifted in the hyperpolarising direction (by 5–19 mV) when the temperature was lowered from 25 °C to 0 °C, this shift could not have contributed significantly to the observed decrease in sodium current because a large negative prepulse was used to remove any inactivation. Finally, any shift in the  $P_{Na}(E)$  curve was small



**Fig. 2** Arrhenius plot of maximal sodium conductance  $\bar{g}_{Na}$  (a) and of time constant of inactivation  $\tau_h$  (b) for frog and rabbit nodes. The data obtained from five rabbit and six frog nodes were normalised in each experiment by finding a least squares fit to the data points between 18 and 12°C and scaling all experimental points for that node so that the conductance at the mid-points of the lines (the expected value at 15°C) were 0.27  $\mu S$  and 0.25  $\mu S$  for the rabbit and the frog nodes, respectively, in panel a, and the corresponding times were 1.4 ms and 1.9 ms for the rabbit and the frog, respectively, in panel b. These values were chosen because they were the actual average values for  $\bar{g}_{Na}$  and  $\tau_h$  obtained experimentally. Each point is the average of 2–9 observations for  $\bar{g}_{Na}$  and 2–19 observations for  $\tau_h$ . The error bars drawn through each point ( $\pm 1$  s.e.m.) are usually smaller than the symbols. The rate of fibre rundown was slow compared with the rate of cooling and was not corrected for. However, rewarming took about 5 times as long leading to a hysteresis in the  $\bar{g}_{Na}$  but not the  $\tau_h$  curves. Two rewarming experiments are included in the mammalian  $\tau_h$  curve.

or absent ( $< \pm 10$  mV) and so could not have caused any substantial variation in current size with temperature since the test pulse of 60–75 mV was in the potential range in which the  $P_{Na}(E)$  curve had already reached a plateau, at both 25°C and 0°C.

The temperature-dependent transition reported here for the conductance of the sodium channel of mammalian myelinated nerve thus resembles the temperature-dependent transition already reported for the acetylcholine-activated channel conductance<sup>6,7</sup> and for other membrane proteins where activity, transport or mobility within the lipid have been assayed as a function of temperature<sup>1</sup>; it also resembles the transition found in frog nerve and muscle<sup>8</sup>. A change in membrane fluidity is thought to occur at these transition temperatures. It is possible that the same mechanism underlies the marked temperature dependence of the various functional parameters of sodium channel reported here for the rabbit node of Ranvier. Alternatively, a conformational change in the channel protein might

have occurred. The fact that there seems to be little or no discontinuity in the electrical parameters of the frog node of Ranvier in the present study may reflect species differences in the lipid composition of the membrane, or differences in the sodium channels themselves. Interestingly, in the rabbit nerve the transition temperature region is about the same for  $\tau_h$  and  $\bar{g}_{Na}$ , suggesting that the molecular structures regulating  $\bar{g}_{Na}$ , and those controlling the gating process, are surrounded by a similar lipid environment<sup>6</sup>. The nature of the marked reduction of  $\bar{g}_{Na}$  at low temperature is unclear; it may result either from a temperature-dependent block of the sodium channels or from a reduction in the single channel conductance. These alternatives might be distinguished by noise analysis experiments. If the transition temperature reported here is related to a phase change in the lipid surrounding the channel, then it might be possible to alter this temperature using agents that modify membrane fluidity.

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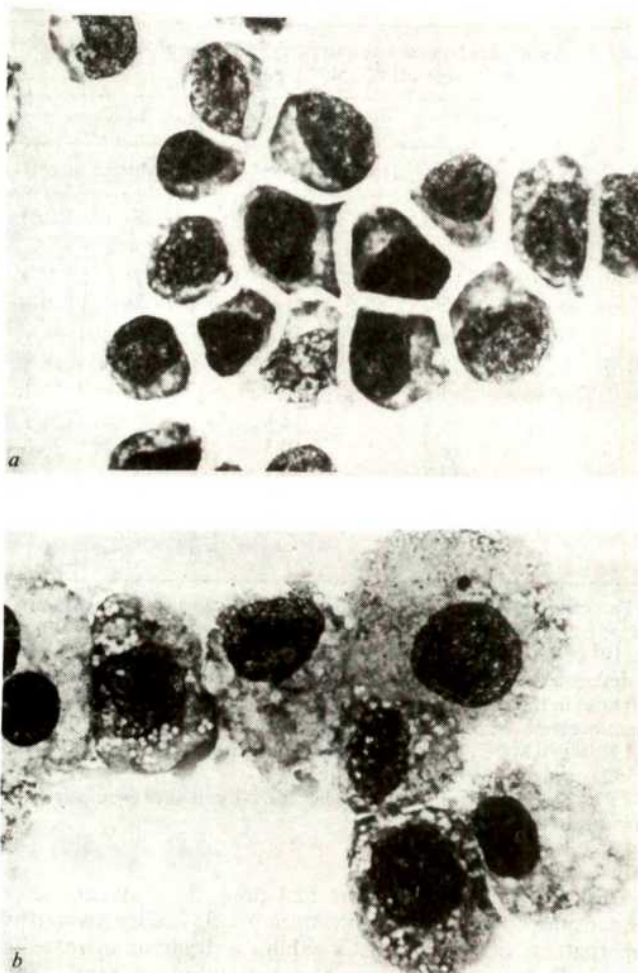
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## In vitro activation of a human macrophage-like cell line

SEVERAL permanent murine macrophage-like cell lines exhibiting various macrophage-associated effector functions have been described<sup>1–3</sup>, but establishment of permanent human macrophage cell lines has been much more difficult<sup>4</sup>. Recently Sundström and Nilsson<sup>5</sup> reported the establishment of a human histiocytic lymphoma cell line, U937, with macrophage characteristics. This line was further adapted to rapid growth *in vitro* by Lachman *et al.*<sup>6</sup>. During studies of major histocompatibility complex antigens on a number of human lymphoid cell lines we failed to detect Ia-like antigens (HLA-DR) on U937. Because these alloantigens have been reported to occur on early myeloid cells<sup>7</sup> and might therefore be regarded as differentiation antigens of stem cells, an attempt was made to produce differentiation and expression of new cell surface molecules on U937. Procedures similar to those used to produce differentiation of myeloid cell lines<sup>8</sup> and promote growth of macrophages in culture were tried. Although U937 cells failed to express Ia-like antigens they underwent remarkable morphological and functional changes. We report here that U937 can be activated by supernatants from human mixed lymphocyte cultures (MLC). Although this activation takes several forms, the findings reported here show marked augmentation of antibody-dependent cellular cytotoxicity (ADCC) against erythroid and tumour target cells.

Figure 1 presents photomicrographs of normal U937 (Fig. 1a) and activated U937 cells (Fig. 1b). Activation markedly alters





**Fig. 1** Cyto-centrifuge preparations of unactivated (a) and activated (b) U937 cells. Human macrophage-like line U937 was adapted to continuous growth in suspension culture independent of monolayer cells or their supernatants<sup>6</sup>. Generation time was 16–20 h. Unactivated cells (a) are grown in RPMI-1640 supplemented with 10% FCS. Activated cells (b) were cultured for 20 h in normal culture medium supplemented with 30% conditioned medium obtained in a one-way MLC on day 6 of culture. The CM used for the experiments presented in this paper used peripheral mononuclear cells from donor DS and an allogeneic B-cell line JoKo (irradiated) as stimulator. Wright stain,  $\times 672$  magnification.

the appearance of U937 cells: the cells become larger, the cytoplasm more vacuolated and the membrane more villous. In culture the activated cells are more adherent and have increased intensity of nonspecific esterase staining (not shown).

One of the principal functions of macrophages is their ability to mediate ADCC<sup>1–3</sup>. We therefore chose the ADCC assay to measure differences in activity of U937 macrophage-like cells. The ADCC data of five representative experiments are presented in Table 1. Unactivated U937 cells exhibited varying levels of ADCC activity against chicken red cell targets modified with TNP (CRC-TNP), as represented in the minimal levels of activity in experiments 1, 2, 4 and 5a, with higher levels in experiment 3. The reason for the fluctuating baseline ADCC levels of normal unactivated U937 is not clear. This variability may be caused by cell-cycle dependence of activity or 'nonspecific' activation by fetal calf serum (FCS) components. In contrast, U937 cultured with supernatants of human MLCs (conditioned medium, CM; see legend to Table 1), consistently showed increased activity in ADCC against CRC-TNP targets. This strong activity of activated U937 was not restricted to CRC-TNP targets; similar results were observed using sheep red cells modified with TNP (SRC-TNP) (Table 1, experiment 5b). Neither activated nor unactivated U937 were cytotoxic against

**Table 1** Antibody-dependent cellular cytotoxicity of activated U937 cell line against erythroid target cells

Expt	Activating* supernatant	ADCC/CRC-TNP % Specific lysis			
		8:1	4:1	2:1	1:1
1	None	8.5	5.4	2.5	1.3
	CM	57.1	47.1	33.8	18.7
2	None	4.1	1.7	0.8	–0.8
	CM	56.5	54.1	46.8	29.6
3	None		21.4	11.9	8.5
	CM		54.2	42.2	31.9
4	None	1.9	0.8	0.0	0.1
	CM	31.8	19.7	11.7	5.8
	JoKo†	4.4	2.4	2.6	0.8
	DS‡	6.6	4.2	1.9	0.8
5a	None	1.3	0.9	0.5	0.5
	CM	41.1	33.2	24.5	15.2
ADCC/SRC-TNP					
5b	None	5.1	1.3	1.6	–0.5
	CM	18.9	12.2	10.9	11.4

The effect of U937 activation on ADCC against erythroid target cells. ADCC was tested in a 2-h <sup>51</sup>Cr release assay against  $3 \times 10^4$  chicken red cells (CRC) or sheep red cells (SRC) modified with 2,4,6-trinitrobenzene sulphonic acid (TNP) and coated with rabbit anti-TNP antibodies, a method previously described<sup>1</sup>. The <sup>51</sup>Cr released in such an assay was due only to extracellular killing<sup>2</sup>. Assays were carried out in triplicate and s.e. did not exceed  $\pm 5\%$ . Spontaneous release of the targets in medium alone ranged between 0.5 and 2.0%. Since insignificant ADCC activity was shown by unactivated or activated U937 cells in the absence of anti-TNP antibodies, the results presented here show only the killing in the presence of anti-TNP antibodies.

\* Conditioned medium (CM) was obtained as described in Fig. 1.

† CM from irradiated JoKo (B-cell line) cultured alone.

‡ CM from peripheral mononuclear cells of individual DS cultured alone.

CRC-TNP or SRC-TNP in the absence of sensitising antibodies (data not shown).

To assess the extent to which product(s) of MLC supernatants were responsible for U937 activation, control experiments using supernatants of the responder cells or the irradiated stimulator cells of the MLC grown alone, were performed. Culturing U937 with CM from cultures of the responder alone (experiment 4—DS) or irradiated stimulator cells of the MLC (experiment 4—JoKo) resulted in low ADCC activity similar to that by unactivated cells (experiment 4).

The possibility that CM could enhance ADCC if present in the assay by a carry-over mechanism was also tested. CM added to

**Table 2** Phagocytosis of erythroid target cells by activated U937

Activating supernatant	Anti-TNP	% Phagocytosis				
		Expt 1	Expt 2	Expt 3	Expt 5a	Expt 5b
None	–	8.3	10.3	6.6	–0.8	–3.2
None	+	12.8	12.2	25.7	0.6	3.4
CM	–	7.9	10.5	7.7	–0.9	–0.8
CM	+	17.9	29.1	26.1	10.2	39.9

The effect of U937 activation on phagocytosis of erythroid target cells. Phagocytosis was determined by measuring <sup>51</sup>Cr of the endocytosed erythrocytes (CRC-TNP and SRC-TNP) by U937, according to the method of Walker and Demus<sup>2</sup>. The phagocytosis experiments presented here correspond to the ADCC experiments with the same number as shown in Table 1. Therefore, experiments 1, 2, 3 and 5a were done with CRC-TNP, whereas SRC-TNP were used for experiments 5b. The E:T ratio chosen for the phagocytosis experiments was 8:1. Groups without anti-TNP antibodies added represent Fc-independent phagocytosis, whereas groups with anti-TNP antibodies reflect Fc-dependent phagocytosis ('immune phagocytosis').

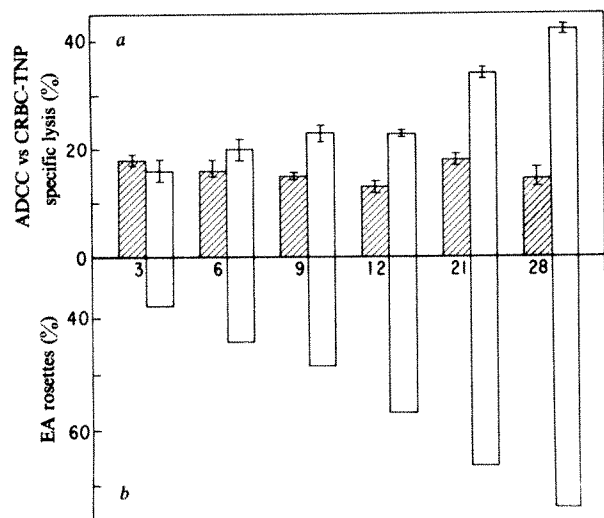
unactivated U937 cells during the 2-h ADCC assay, however, did not alter their activity (data not shown).

The activation of U937 is rapid. Enhanced ADCC against CRC-TNP was seen 6 h after addition of CM and reached a twofold increase after 28 h (Fig. 2a). In another study stimulation was measured as early as 3 h, reaching a peak after 3–5 d. Cultures fed with normal medium gradually lost activity after 3 weeks.

As ADCC is Fc receptor (FcR) dependent it was of interest to test whether activation of U937 correlated with FcR expression by these cells. A steady increase in the number of FcR+ cells was indeed detected by rosetting with antibody-coated erythrocytes (EA rosettes) as shown in Fig. 2b. A doubling in the number of FcR+ cells occurred during the first 28 h. By using aggregated  $^{125}$ I-IgG, as an independent measure of FcRs, a similar rising pattern was seen during activation. These data (not shown) clearly establish a direct relationship between the number of FcR+ cells in the activated U937 population and Fc-dependent phenomena such as ADCC.

Phagocytosis by activated U937 was enhanced in several experiments (Table 2, experiments 2, 5a and 5b) while in others (experiments 1 and 3) it did not exceed phagocytic levels of the unactivated U937. In all cases a greater percentage of target cells was phagocytosed when antibodies were present (antibody-dependent) than when they were absent (antibody-independent).

Although human peripheral blood monocytes have repeatedly been shown to mediate ADCC to several red cell targets<sup>9</sup>, their role in ADCC against tumour targets is controversial. Many studies suggest that antibody-coated tumour targets are killed by K cells<sup>10,11</sup>, whereas others ascribe this effector function to both K cells and macrophages<sup>12–14</sup>. Unactivated U937 cells have no ADCC activity against neoplastic cells. However, *in vitro* activation of the cell line as described in Fig. 1 resulted in significant and reproducible cytolysis of a variety of neoplastic cells (see Table 3). A wide spectrum of lymphoblastoid cell lines (LCL) and a myeloid line were lysed by activated U937 in a 4–6.5 h assay at effector:target ratios varying from 12.5:1 to 100:1. Target cells of human (HSB, MOLT4, SB and K562) and murine (EL4 and RL $\delta$ 1) origin with different surface characteristics were found to be sensitive targets.



**Fig. 2** Time kinetics of U937 activation and its effect on ADCC and FcR expression. *a*, ADCC of unactivated  $\square$  and activated  $\square$  U937 was measured in a 2-h  $^{51}$ Cr release assay against CRC-TNP (see Table 1). U937 cell line was activated with 30% CM and samples were tested after 3, 6, 9, 12, 21 and 28 h of cultivation. *b*, FcR+ cells in the activated U937 population  $\square$  were measured by their ability to form rosettes with ox red cells (ORC) sensitised with rabbit 7S anti-ORC antibodies. FcRs were expressed in 30–40% of unactivated U937 cells (data not shown).

**Table 3** Antibody-dependent cellular cytotoxicity of activated U937 against tumour target cells

Expt	E:T ratio	% Specific lysis					
		Human targets*			Murine targets†		
		HSB	MOLT4	SB	K562	EL4	RL $\delta$ 1
1	100:1	22.6	13.7		4.8	29.9	
	50:1	19.0	18.4		5.3	26.0	
	25:1	16.0	13.5		6.3	26.7	
2	50:1	8.0		7.0		6.5	26.7
	25:1	5.9		3.0		5.4	18.8
	12:1	4.8		4.0		2.6	11.1
3	50:1	45.3		14.5		21.4	24.2
	25:1	46.2		10.5		17.2	26.8
	12:1	33.8		9.1		15.6	14.2
4	50:1	52.9			7.5	40.5	
	25:1	43.8			6.1	36.8	
	12:1	37.8			2.2	25.8	

The effect of U937 activation on ADCC against nucleated tumour target cells. ADCC was tested in a 4–6.5 h  $^{51}$ Cr release assay against  $1 \times 10^4$  target cells TNP-modified and coated with anti-TNP antibodies as described above (see Table 1). Controls of unactivated U937 and cell mixtures in the absence of anti-TNP antibodies were negative and were therefore omitted. Activation was performed as described in Fig. 1.

\* HSB and MOLT 4 are leukaemic T-cell lines, SB is a B-cell line, and K562 is a myeloid leukaemia cell line.

† EL4 and RL $\delta$ 1 are T-cell lymphomas. All cell lines were carried as suspension cultures *in vitro*.

This study describes for the first time, the activation of a human macrophage-like cell line *in vitro*. U937 cells activated by supernatants of human MLCs exhibit a dramatic increase in their ADCC activity towards a variety of erythroid and nucleated-neoplastic targets. The activation is rapid and the marked morphological changes which occur are accompanied by an increase of FcR-bearing U937 macrophage-like cells. These results are in accordance with those of Shaw *et al.*<sup>13</sup> who have demonstrated ADCC activity by peripheral monocytes against nucleated target cells although McDonald *et al.*<sup>10</sup> and Nelson *et al.*<sup>11</sup> have reported activity mediated exclusively by K cells in their systems. The demonstration that a human macrophage-like cell line mediates ADCC against erythroid and neoplastic cells, and that this activity can be augmented, or in most cases induced in culture, will allow further investigation into the mechanism of activation and cytolysis by human macrophages. Biochemical studies of the activating molecule(s) are in progress.

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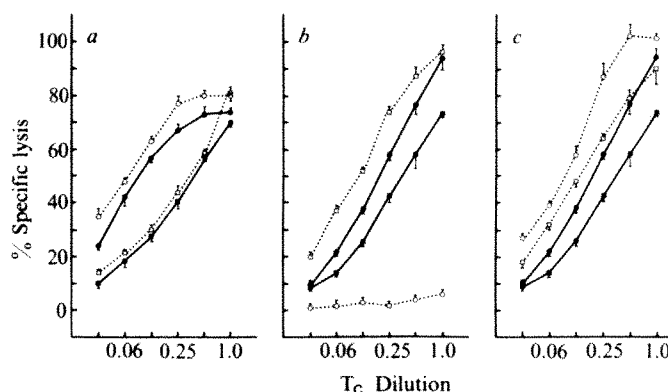
## Specific depletion of alloreactive cytotoxic lymphocyte precursors

THE major barrier to replacement therapy in various clinical immunodeficiency states is the likelihood that the transferred immunocompetent T lymphocytes would react against alloantigens of the recipient and produce graft-versus-host (GVH) disease<sup>1</sup>. The logical resolution of this problem would be to delete or inactivate those clones of T cells which are specifically coded to react against the histocompatibility antigens of the recipient tissues. The remaining cell population could then fully reconstitute the recipient without danger of GVH reactions. Specific clonal deletion of this kind has been obtained in experimental animal situations by pre-circulation through a third-party host<sup>2,3</sup> or by induction of specific anti-idiotypic immunity<sup>4</sup>. These studies show the validity of this approach but are technically complicated and have limited application. A simpler method of obtaining this result has been sought based on the following strategy. To reconstitute a strain A mouse by transfer of lymphocytes from an allogeneic strain B, the strain B lymphocytes are first incubated on a monolayer of cells of strain A. Those clones which recognise the strain A alloantigens should adhere to the monolayer; the non-adherent populations of cells should now be unable to react against alloantigens of strain A but should still respond to alloantigens of other haplotypes. Although this kind of technique has been widely effective in removing fully activated cytotoxic lymphocytes (T<sub>c</sub>)<sup>5-9</sup>, its use to deplete alloreactive T<sub>c</sub> precursors has given variable results<sup>8,10-16</sup>. Significant depletion of precursors has been reported primarily when the non-adherent cells have been assayed *in vivo*<sup>13-16</sup>, but this has been claimed to be due to change in the responder cells, not depletion, as the same population assayed by *in vitro* generation of T<sub>c</sub> shows no evidence of depletion<sup>16</sup>. We present here a simple depletion method developed from our finding that when splenic lymphocytes are incubated for a few hours before being formed into a monolayer, they can effectively absorb alloreactive T<sub>c</sub> precursors even when assayed by the sensitive *in vitro* assays.

Figure 1 shows the results of experiments in which BALB/c responder cells were absorbed on monolayers of spleen cells constructed by attachment to Petri dishes coated with poly-L-lysine (PLL); the non-adherent responder cells were then

cultured with X-irradiated stimulator cells and after 5 d assayed for the presence of T<sub>c</sub>. Absorption on monolayers of fresh C57BL/10 (B10) spleen cells failed to reduce the BALB/c T<sub>c</sub> response to stimulation with B10 alloantigen (Fig. 1a), in agreement with reports of similar failures<sup>8,16</sup>. In contrast, absorption on monolayers constructed with B10 spleen cells which had been preincubated at 37 °C for 4 h abolished the response to B10 alloantigens (Fig. 1b). The specificity of the absorption is shown by the ability of the same population of non-adherent BALB/c cells to mount a normal T<sub>c</sub> response to B10.BR alloantigens, and further by the failure to deplete the response to B10 or B10.BR alloantigens when the absorbing monolayer was composed of preincubated BALB/c spleen cells (Fig. 1c). The latter experiment also demonstrates a consistent finding that absorption on a monolayer of preincubated cells commonly increases the T<sub>c</sub> response to haplotypes other than that of the absorbing monolayer, but this is not attributable to carry-over of monolayer cells. In this experiment, and those described below, the numbers of non-adherent cells recovered after incubation on the absorbing monolayer were consistently 97-102% of the number of responder cells applied, and by immunofluorescence tests with anti-H-2 antisera, cells of monolayer haplotype comprised only 3-6% of this population.

Table 1 shows that the technique is applicable to other strains. B10.BR spleen cells were specifically depleted of T<sub>c</sub> precursors



**Fig. 1** Effect of pre-incubation on the ability of splenic monolayers to deplete T<sub>c</sub> precursors. Monolayers for absorption were constructed by attaching  $\sim 2 \times 10^7$  spleen cells to polystyrene Petri dishes (50  $\times$  13 mm, Sterilin no. 122) previously coated with poly-L-lysine (Sigma)<sup>6</sup>. The spleen cells, prepared as previously described<sup>17</sup>, were used immediately or after 4 h incubation in 75-cm<sup>2</sup> flasks (Falcon no. 3024) containing 30 ml Eagle's minimum essential medium plus non-essential amino acids, supplemented with 10% v/v fetal calf serum (FCS) and  $7 \times 10^{-10}$  M 2-mercaptoethanol (MEM.FCS) at 37 °C with 10% CO<sub>2</sub> in air (pre-incubated spleen cells). The monolayers were washed twice in phosphate-buffered saline, overlaid with 2 ml of MEM.FCS and incubated for 5 min at room temperature before addition of responder cells ( $2 \times 10^7$ ) in 2 ml MEM.FCS. The dishes were centrifuged at 100g for 5 min in a prewarmed MSE Mistral 2L centrifuge and incubated for 1 h at 37 °C with 10% CO<sub>2</sub> in air. The non-adherent responder cells were recovered from the dishes by brief agitation, washed once, and  $2 \times 10^7$  resuspended in 9 ml MEM.FCS and transferred to a 25-cm<sup>2</sup> flask (Falcon no. 3013). Control cultures containing  $2 \times 10^7$  unfractionated responder cells in 9 ml MEM.FCS were similarly prepared. To each flask  $2 \times 10^7$  X-irradiated (1,000 R) B10 or B10.BR stimulator spleen cells were added in 1 ml MEM.FCS. All cultures were prepared in duplicate and incubated at 37 °C for 5 d with 10% CO<sub>2</sub> in air. Cytotoxicity was determined using a microassay previously described<sup>18</sup>. Duplicate cultures were pooled, washed and resuspended in 0.6 ml RPMI 1640 supplemented with 10% v/v FCS. All T<sub>c</sub> populations were titrated against <sup>51</sup>Cr-labelled tumour target cells of both stimulator haplotypes. The results obtained were expressed as % lysis  $\pm$  s.d. of triplicate determinations. Per cent specific lysis represents the % lysis minus the spontaneous release of label by target cells incubated with medium alone. Absorption of BALB/c (H-2<sup>d</sup>) responders on: a, fresh B10 (H-2<sup>b</sup>) monolayers; b, preincubated B10 monolayers; c, preincubated BALB/c monolayers. Comparison of the cytotoxicity generated against the homologous tumour target cell, when non-adherent responder cells were stimulated with B10 ( $\circ \cdots \circ$ ), or B10.BR (H-2<sup>k</sup>,  $\square \cdots \square$ ) as an unrelated haplotype, with unfractionated controls similarly stimulated (B10  $\bullet \cdots \bullet$ , B10.BR  $\blacksquare \cdots \blacksquare$ ). To eliminate any effect of storage of the responder cell populations during the 4-h preincubation of monolayer cells, a separate, fresh responder population was prepared for those experiments in b and c; hence, the unabsorbed control values differ slightly in a from those in b and c.

**Table 1** The ability of pre-incubated B10 and BALB/c monolayers specifically to deplete B10.BR T<sub>c</sub> precursors reactive towards H-2<sup>b</sup> or H-2<sup>d</sup> alloantigens

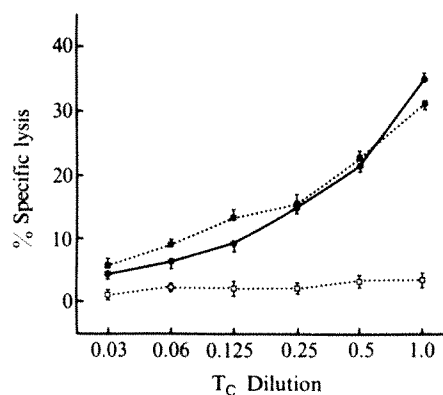
Responder	Pre-incubated monolayer	Stimulator	Cytotoxic titre H-2 <sup>b</sup> target	H-2 <sup>d</sup> target
B10.BR	—	B10	20.0	8.4
B10.BR	—	BALB/c	<1	55.7
B10.BR	B10	B10	<1	16.8
B10.BR	B10	BALB/c	<1	250.0
B10.BR	BALB/c	B10	50.1	5.0
B10.BR	BALB/c	BALB/c	<1	3.3

B10.BR responder cells were absorbed on monolayers of B10 or BALB/c preincubated spleen cells as described in Fig. 1. Non-adherent cells, or unfractionated responders as controls, were stimulated by either B10 or BALB/c alloantigen. The cytotoxic titre for a given T<sub>c</sub> population was calculated as the reciprocal of the highest dilution of the T<sub>c</sub> population which gave 15% specific lysis of the target concerned.

against H-2<sup>d</sup> or H-2<sup>b</sup> alloantigens by exposure to monolayers of preincubated BALB/c or B10 spleen cells, respectively. Similar results were obtained when CBA responder cells were used. The technique described thus seems applicable to several strain combinations.

Two further sets of experiments, by eliminating alternative explanations, strongly supported the suggestion that the results obtained are attributable to binding of T<sub>c</sub> precursors to the absorbing monolayer. First, 10<sup>7</sup> B10.BR cells which remained non-adherent after exposure to a monolayer of 4-h incubated B10 cells were added to an equal number of untreated, normal B10.BR spleen cells and cultured for 5 d with X-irradiated B10 stimulator cells. For comparison, a mixture of 10<sup>7</sup> X-irradiated and 10<sup>7</sup> normal B10.BR responder cells were cultured in the presence of X-irradiated B10 stimulator cells. Control cultures containing 10<sup>7</sup> non-adherent and 10<sup>7</sup> X-irradiated B10.BR responder cells were similarly stimulated. The results in Fig. 2 show that the non-adherent B10.BR cells did not significantly suppress ( $P > 0.3$ ) the response of normal B10.BR spleen cells to stimulation by B10 alloantigens. These results argue against the reduction in T<sub>c</sub> generation achieved by pre-exposure to an absorbing monolayer being attributable to induction of a suppressor population, or to blockage of the response by B10 cells or cell debris carried over from the monolayer.

Second, the reduction in T<sub>c</sub> generation is not due to depletion of a proliferative, helper population which has been claimed to be necessary for T<sub>c</sub> generation<sup>17</sup>. This was established by comparing <sup>3</sup>H-thymidine incorporation and T<sub>c</sub> generation of BALB/c cells which had been exposed to an absorbing monolayer of 4-h incubated B10 cells. The results in Table 2 show that, as expected, the non-adherent BALB/c cells were unable to mount a cytotoxic response when stimulated by B10 alloantigen but continued to show a normal T<sub>c</sub> response to B10.BR alloantigen. However, the proliferation of the non-adherent BALB/c cells in response to B10 stimulation has not been impaired; thus, the B10 monolayer has not depleted this responding, helper-type population. This result has been confirmed in four other experiments. The controls in this experiment show that the non-adherent cells show no significant proliferation in the absence of any added stimulus, implying that significant carry-over of B10 antigen or of B10 cells capable of proliferation (or stimulation) has not occurred. This agrees with the immunofluorescence tests cited above which showed only a very low release of monolayer cells. Further corroboration that our results are attributable to specific binding of the T<sub>c</sub> pre-



**Fig. 2** Failure of B10.BR responder cells, non-adherent to pre-incubated B10 monolayers, to suppress the cytotoxic response of normal, unfractionated B10.BR responder cells to B10 alloantigens. 10<sup>7</sup> B10.BR responder cells which remained non-adherent after exposure to a monolayer of 4-h incubated B10 cells, were added to an equal number of untreated, normal B10.BR spleen cells (■ · · · ■), and cultured for 5 d with X-irradiated B10 stimulator cells. Controls containing 10<sup>7</sup> X-irradiated (1,000 R) and an equal number of normal B10.BR responder cells (● · · · ●), and 10<sup>7</sup> non-adherent and 10<sup>7</sup> X-irradiated B10.BR responder cells, (□ · · · □) were similarly stimulated. Cytotoxicity was determined by titration of the T<sub>c</sub> populations against <sup>51</sup>Cr-labelled H-2<sup>b</sup> tumour target cells.

cursors by the absorbing monolayer comes from preliminary experiments in which T<sub>c</sub> against the monolayer haplotype were generated by culturing the adherent cells.

In conclusion, the method outlined here of absorption on a monolayer of preincubated lymphocytes gives extensive haplotype-specific depletion of T<sub>c</sub> precursors when subjected to the sensitive assay of *in vitro* induction of cytotoxic lymphocytes and has so far been successful in tests using various responder-stimulator combinations of eight mouse strains. The success of this method, in contrast to the variable results achieved in earlier reports, is due to the use of preincubated lymphocytes, which seem to form a more efficient absorbing monolayer. This could be due to a change in antigen presentation<sup>24</sup>, which increases the binding affinity of T<sub>c</sub> precursors, or to an increased binding of the absorbent monolayer cells to the PLL-plastic, preventing dislodgement of any complex of bound T<sub>c</sub> precursors and absorbing cell, or to a combination of both these factors.

Our results suggest two areas for further investigation. First, to define the change(s) induced by the pre-incubation of spleen cells that leads to effective T<sub>c</sub> precursor binding, and second, to investigate whether GVH activity is affected by the removal of T<sub>c</sub> precursors. However, the ability to deplete, and possibly recover, a population of T cells with restricted haplotype alloreactivity should be of immediate value in other areas of immunology, for example, in clarifying the role of major histocompatibility complex products in recognition by T cells<sup>21</sup>, or in evaluating the alternative models which have been proposed for alloreactive T-cell triggering<sup>22-24</sup>.

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**Table 2** Absorption of BALB/c responder cells on pre-incubated B10 monolayers fails to reduce the proliferative response of the non-adherent responder cells towards B10 alloantigens

Responder	Preincubated monolayer	Stimulator	Proliferative response ( <sup>3</sup> H c.p.m.)	Cytotoxic titre H-2 <sup>b</sup> target	H-2 <sup>k</sup> target
BALB/c	—	B10	31,697 ± 2,786	16.7	2.0
BALB/c	—	B10.BR	43,967 ± 2,756	1.0	20.0
BALB/c	B10	B10	34,987 ± 2,625	<1	1.7
BALB/c	B10	B10.BR	49,457 ± 3,233	<1	21.3
BALB/c	B10	BALB/c	5,572 ± 416		
BALB/c	B10	Nil	5,147 ± 330		
BALB/c	—	BALB/c	4,905 ± 487		
BALB/c	—	Nil	4,709 ± 349		
Nil	—	BALB/c	90 ± 31		

BALB/c responder cells were absorbed on monolayers of pre-incubated B10 spleen cells as described in Fig. 1. 10<sup>7</sup> Non-adherent cells, or 10<sup>7</sup> unfractionated responders as controls, were stimulated by 10<sup>7</sup> X-irradiated (1,000 R) B10, B10.BR or BALB/c stimulators, or none (nil), in 5 ml medium. To determine the proliferative response, duplicate 0.3-ml samples of each culture, after 3 d incubation, were transferred to a microtitre tray (Sterilin no. M29ARTL). Following centrifugation at 100g for 5 min, approximately half the supernatant was discarded and 25 µl of medium containing 0.5 µCi of <sup>3</sup>H-thymidine was added to each well. After incubation in a humidified box for 18 h with 10% CO<sub>2</sub> in air, cultures were transferred to microtubes and collected according to the method of Smart *et al.*<sup>20</sup>. Results obtained were expressed as <sup>3</sup>H c.p.m., and represent the mean ± s.d. of quadruplicate determinations. Cytotoxicity was determined as described in Fig. 1 after 5 d incubation.

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## Reciprocal expansions of idiotypic and anti-idiotypic clones following antigen stimulation

It has been postulated by Jerne that a network of idiotypic and anti-idiotypic interactions between lymphocytes may constitute a principal form of immune regulation<sup>1,2</sup>. This network would control and 'remember' the antigen-induced activation of a particular (idiotypic) clone through its idiotypic or paratopic interactions with regulatory clones. These latter clones would in turn be regulated in the same fashion, creating what Jerne has called a 'web of V-domains' (ref. 2). Current experimental evidence strongly supports the concept of idio-type-directed regulation and also documents phenotypic complexity within the anti-idiotypic response; for example, anti-idiotypic cells (and/or their products) may help or suppress the idiotypic response<sup>3–8</sup>. However, this apparent complexity does not diminish the importance of Jerne's basic premise that reciprocal activation between idiotypic and anti-idiotypic clones could establish an equilibrium system capable of regulating the immune response<sup>9–11</sup>. We present here evidence for oscillatory behaviour in the population dynamics of a single, antigen-stimulated clone and for the reciprocal behaviour of the specific anti-idiotypic (idiotype binding) clones.

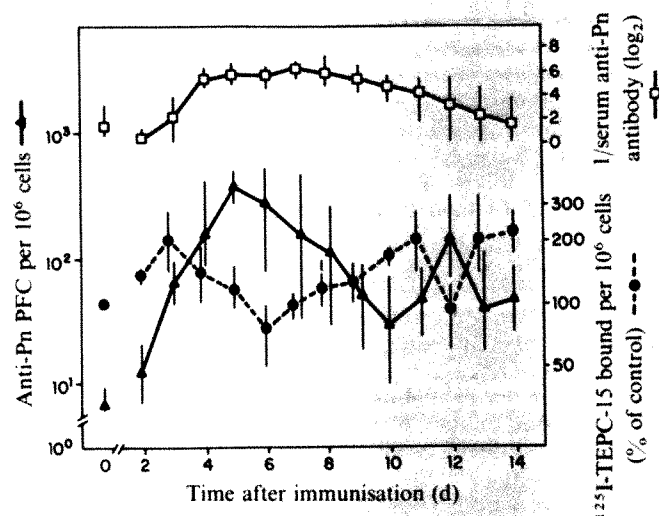
Our studies used the T-15/phosphorylcholine system in BALB/c mice. These mice, immunised with a vaccine of strain R36a *Streptococcus pneumoniae* (Pn), produce an essentially monoclonal, T-independent antibody response. The idiotype (T-15) of this anti-Pn antibody is shared with the myeloma protein secreted by the BALB/c plasmacytoma, TEPC-15. Both are specific for the hapten phosphorylcholine (PC)<sup>12</sup>. The kinetics and magnitude of the response of the T-15-bearing cells in the spleen was determined in two ways: (1) by the binding of radioactive [<sup>14</sup>C]PC and (2) by counting anti-Pn antibody plaque-forming cells (PFC) in agarose using R36a polysaccharide-coated sheep red blood cells (RBC) as indicator cells<sup>13</sup>. Both assays gave similar results; only the PFC numbers will be presented here. Serum antibody to Pn was measured by passive haemagglutination of antigen-coated sheep RBC. Changes in those clones recognising the T-15 idiotype (that is, the anti-idiotypic clones) were determined by the binding of the radiolabelled <sup>125</sup>I-myeloma protein, TEPC-15, on spleen cells. As a control, we used: (1) <sup>125</sup>I-MOPC-315 myeloma protein which has the same isotype (IgA) as TEPC-15 but which has both different idiotype and binding specificity; and, (2) <sup>125</sup>I-McPC-603, an IgA myeloma protein that binds PC but does not bear the T-15 idiotype<sup>14</sup>.

The time course of the idiotypic (T-15) and anti-idiotypic (T-15 binding) clonal responses after stimulation by Pn was visualised by the sequential immunisation of groups (3–4) of BALB/c mice (age- and sex-matched). Daily, for 14 d, a group of mice was immunised by an intraperitoneal injection of 20 µg of Pn vaccine. Thus, each group represented a unique stage of the anti-Pn response. On the 15th day, all immunised mice and a group of unimmunised mice were killed, and their spleens removed and disrupted into single cell suspensions. These cells

were washed three times in media and finally resuspended to a concentration of 10<sup>7</sup> viable lymphocytes per ml. Duplicate aliquots (10<sup>6</sup> cells in 0.1 ml) of these suspensions were then assayed as described above. This experimental schedule allowed mice at different stages of the anti-Pn response to be assayed simultaneously and using the same reagents. However, not more than three to four mice per interval could be handled. Therefore, to ensure the reproducibility of the data, we repeated the experiment four times, obtaining similar results in each trial.

As shown in Fig. 1, the accumulation of specific anti-Pn PFC in the spleen after a single injection of the antigen followed a cyclical pattern: a broad peak on day 5 (≈ 700 PFC per 10<sup>6</sup> splenocytes) was followed by a second, sharper peak on day 12 (≈ 200 PFC per 10<sup>6</sup> splenocytes). Specific binding of the hapten (PC) to the spleen cells coincided with the curve of the PFC, usually reaching a maximum 1 d before the PFC peak (data not shown). However, serum anti-Pn antibody titres did not reflect the cyclical pattern of splenic PFC; titres were highest around day 8 and then declined steadily.

The specific binding of <sup>125</sup>I-TEPC-15 by spleen cells from the immunised mice also underwent cyclical changes that seemed to be out of phase with the PFC curve (Fig. 1). Thus, the relative binding of T-15 (per cent of the control) rose to a maximum



**Fig. 1** Groups of three to five BALB/c mice were injected intraperitoneally with 20 µg of Pn vaccine at 2–14 d before removal of their spleens. Aliquots containing 10<sup>6</sup> viable splenic lymphocytes were assayed for anti-Pn PFC (▲) and specific binding of <sup>125</sup>I-TEPC-15 myeloma protein (●). Serum, collected at the time of splenectomy, was assayed for anti-Pn antibody by passive haemagglutination of antigen-coated sheep RBC (□). Each symbol represents the mean of four experiments (12–15 mice). The vertical bars indicate the range of those experiments. For determination of <sup>125</sup>I-TEPC-15 binding, ascitic fluid from BALB/c mice bearing the plasmacytomas TEPC-15, MOPC-315 or McPC-603 was recovered and purified by (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> precipitation and gel (Sephadex G-200) filtration. Each purified protein was tested for its capacity to haemagglutinate Pn- or TNP-coated sheep RBC. Iodination was with radioiodinated Bolton-Hunter reagent (NEN), 5 mg protein per mCi <sup>125</sup>I. Iodinated proteins were equivalent to non-labelled proteins in their ability to haemagglutinate. To determine the optimal concentration of radioligand, spleen cells from BALB/c mice injected with TEPC-15 protein<sup>19</sup> and then allowed to rest for 2 months were incubated with 10-fold dilutions of <sup>125</sup>I-TEPC-15 or <sup>125</sup>I-MOPC-315 0.1–100 µg protein. Saturation occurred at ≈ 1 µg for both proteins. Inhibition of binding (68–75%) occurred in the presence of 100 µg cold homologous ligand but not with the heterologous ligand or bovine serum albumin. To measure T-15 binding, duplicate tubes containing 10<sup>6</sup> cells in 0.1 ml of ice-cold Hank's balanced salt solution containing 2% FCS were incubated at 4 °C for 10–12 h with ≈ 1 µg (0.05 ml) of labelled TEPC-15, MOPC-315 or McPC-603. The cells were then washed three times in cold Hank's containing 10% FCS and the radioactivity of the cell pellet was determined as c.p.m. Variation within duplicates was routinely less than 15%. Specific TEPC-15 binding is defined as: (c.p.m. bound <sup>125</sup>I-TEPC-15) – (c.p.m. bound <sup>125</sup>I-MOPC-315 or <sup>125</sup>I-McPC-603). In any single experiment, specific TEPC-15 binding was computed by the subtraction of only one of the control proteins. Because the c.p.m. of bound MOPC-315 or McPC-603 remains constant (see text) throughout the 14-d experimental period, both serve equally well as specificity controls. The specific TEPC-15 binding is then expressed as per cent of binding by spleen cells from the control group of unimmunised mice (control = 100%).

**Table 1**  $^{125}$ I-TEPC-15 binding in the presence of excess McPC-603\*

	Time (days) after immunisation with Pn							
	0	3	4	6	8	9	10	12
T-15 + McPC-603	100	111	125	86	164	147	127	79
Average T-15 bound	100	190 (120–260)	150 (120–180)	74 (50–100)	110 (90–160)	115 (75–145)	160 (150–195)	90 (60–125)

\* Groups containing three age- and sex-matched BALB/c mice were immunised as described in the text. On day 0 mice were killed and from each group a suspension of pooled spleen cells was made in ice-cold RPMI-1640 media with HEPES buffer (pH 7.3). The cells were washed three times in fresh media and resuspended to a concentration of  $10^7$  viable lymphocytes per ml. Aliquots of 0.1 ml of the appropriate cell suspension were then added to duplicate tubes containing 3  $\mu$ g of  $^{125}$ I-TEPC-15 and 100  $\mu$ g of McPC-603. Cells were incubated overnight at 4 °C and then washed three times in Hank's balanced salt solution containing 10% fetal calf serum (FCS) (pH 7.2). The recovered cell pellets were then counted as described. Binding is expressed as per cent of the day 0 binding value. T-15 binding was measured as described in the text. Binding is expressed as per cent of the day 0 control and the range of four experiments is indicated in parentheses.

(two- to threefold) on day 3, declined steadily until day 6, and then increased to a second maximum by days 10–11, which immediately preceded the second PFC peak (day 12). T-15 binding fell on day 12, then increased again to the third maximum value on days 13–14.

The probe for T-15 binding cells—the radioiodinated TEPC-15 myeloma protein—could bind in at least three ways: by the Fc fragment, by the PC-specific binding site and by the T-15 idiotype. Thus, the relative changes in T-15 binding on splenocytes following Pn immunisation could result from members of Fc receptor-bearing cells or from residual antigen. The MOPC-315 and the McPC-603 myeloma proteins acted as controls for these variables; in particular, the McPC-603 protein has the PC binding paratope and IgA isotype but lacks the T-15 idiotype<sup>14</sup>. The binding of the control probes, MOPC-315 and McPC-603, remained relatively constant ( $\pm 5\%$  of the day 0 value) over the 14-d period following immunisation with Pn. The biphasic pattern of T-15 binding was retained even when cells were incubated with  $^{125}$ I-TEPC-15 in a large excess of McPC-603 protein (Table 1). Thus, it is likely that the T-15 probe predominantly measured anti-idiotypic cells.

Our data on PFC oscillation agree with previous results showing a cyclical pattern of *Escherichia coli* lipopolysaccharide-specific PFC in mice<sup>15</sup> and PFC against human immunoglobulin in rabbits<sup>16</sup>. In the latter study, PFC peaks were observed on days 6 and 12 after immunisation and, as in our experiments, this oscillation was not reflected in the serum antibody levels.

Earlier work from Nisonoff's laboratory has demonstrated the sequential activation of antigen-reactive clones during the immune response<sup>17</sup>. To ensure that the T-15 idiotype dominated both anti-Pn PFC peaks, we carried out a plaque-inhibition assay using a rabbit anti-T-15 antiserum (rendered specific by absorptions with: (1) pooled murine IgM; (2) MOPC-315; and (3) C57BL/6 spleen cells). The addition of this serum at a dilution of 1:500 inhibited both the day 6 and day 12 BALB/c anti-Pn PFC response by  $\geq 80\%$ . The C57BL/6 anti-Pn response was inhibited by  $\leq 9\%$  and the BALB/c anti-TNP response was unaffected.

The increase of T-15 binding by splenic lymphocytes (day 3) suggests an expansion of the anti-idiotypic clones, presumably through cell proliferation. Nonetheless, alternative explanations for the increased binding, such as shedding and passive uptake of T-15 receptors or a trapping of circulating, receptor-bearing lymphocytes in the spleen, cannot be excluded *a priori*. The expansion of the anti-idiotypic clones seems to be cyclical. However, the decrease in T-15 binding observed between the peaks may be partly due to occupation of the receptors by anti-Pn antibody. Because the serum anti-Pn titres do not oscillate, it is more likely that this would result from antibody production *in situ*. Within the micro-environment of the spleen, local concentrations of idiotype antibody might be sufficient to occupy a significant number of receptors on idiotype-binding cells. That the second peak of T-15 binding (day 10) does reflect an expansion of the anti-idiotypic clone rather than a mere unmasking of the receptors for the idiotype is supported by Cosenza's finding of anti-T-15 PFC during the immune response to Pn<sup>18</sup>. These PFC appeared on days 7–8 after immunisation,

following the decline of anti-Pn PFC. We find that at this time the T-15 binding begins to increase and continues to do so until days 10–11; presumably, the binding assay detects cells other than PFC.

Our data demonstrate the periodic expansion of an antigen-activated idiotype clone associated with a reciprocal expansion and diminution of cell-associated, anti-idiotypic (idiotype binding) activity. The pattern is consistent with the hypothesis of a regulatory network based on an idiotype-anti-idiotypic equilibrium. It is tempting to speculate that the out-of-phase expansions of antibody-forming cells and cell-associated anti-idiotypic activity result from the interaction of the idiotype clone with its anti-idiotypic regulators.

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## A possible mechanism for insulin resistance and hyperglycaemia in NZO mice

THE New Zealand obese (NZO) mouse is characterised by obesity, hyperinsulinaemia, insulin resistance and mild glucose intolerance<sup>1–8</sup>. It has been thought of as a model of the adult-type human diabetes mellitus, but neither the genetics of the NZO syndrome nor its basic cause have been elucidated. The metabolic disturbances found in these mice, for example



**Table 1** Effect of NZO serum on insulin binding to normal mouse liver membranes

	Control serum	NZO serum				
		1:2	1:4	1:8	1:16	1:32
<sup>125</sup> I-insulin specifically bound (% of total)	21.5	23.9	8.5	11.6	13.1	17.0

Approximately 100 µg of mouse liver membrane (10-week-old NIH white mice) was exposed to 50-µl dilutions of NZO serum in pooled control serum for 1 h at 24 °C. The membranes were then pelleted in a Beckman Microfuge and washed twice by resuspension in 0.1 M sodium phosphate buffer at 4 °C. <sup>125</sup>I-insulin (50 pg; ~13,000 c.p.m.) in 100 µl of 0.1% bovine albumin-0.1 M sodium phosphate buffer, pH 7.4, was added to the washed membranes, with or without excess unlabelled insulin (10 µg) and the mixtures incubated for 90 min at 15 °C. Membranes were pelleted, washed and counted for <sup>125</sup>I-insulin radioactivity in a gamma counter. Radioactivity bound in the presence of excess unlabelled insulin (nonspecific binding) was subtracted to give specific binding. Results are the means of two experiments each performed in triplicate.

increased rates of lipogenesis<sup>9-12</sup> and defects in insulin secretion<sup>8,13,14</sup>, could well be secondary to chronic hyperinsulinaemia and hyperglycaemia. Because the NZO strain appeared to share a common ancestry with the NZB model of systemic autoimmune disease<sup>1,2,15</sup> we felt that the metabolic syndrome in the NZO mouse might have an immune basis. It was originally suggested that the insulin resistance of NZO mice might be due to an antagonist to the action of insulin<sup>2</sup>. We now report that NZO serum contains a globulin, probably an autoantibody, which inhibits insulin binding and indirectly immunoprecipitates the solubilised insulin receptor.

Initial studies were performed with pooled NZO sera from mice of both sexes older than 12 months. We then developed our own inbred colony from mice derived from the original stock of Bielschowsky and Bielschowsky<sup>1</sup>. The mice had free access to food and water and became obviously obese by 8-10 weeks of age. Orbital sinus blood samples were obtained between 9 and 10 a.m. from 5-20-week-old animals in the post-absorptive state.

The effect of NZO serum on insulin binding was studied by preincubating a source of insulin receptors (NZO, BALB/c or NIH mouse liver membranes, human placental membranes, or cultured IM-9 lymphocytes) with NZO serum serially diluted in control mouse serum (NZW, BALB/c, NIH or CBA mice), followed by washing and measurement of specific <sup>125</sup>I-insulin binding. The preparation of receptors<sup>16,17</sup>, and biologically active <sup>125</sup>I-insulin<sup>18</sup>, and the binding technique<sup>19</sup>, have been described previously. Preincubation of normal mouse liver

**Table 2** Insulin binding to normal mouse liver membranes preincubated with serum from two NZO mice, before and after treatment of each serum with anti-mouse κ antiserum

	<sup>125</sup> I-insulin specifically bound (% of total)	
	Untreated	Treated
Control serum	12.3	12.1
NZO-3	2.5	6.0
NZO-4	5.9	10.3

Serum (75 µl) from male NZO mouse no. 3, female NZO mouse no. 4 and a normal NIH mouse pool was incubated with 75 µl of rabbit anti-mouse κ antiserum (Miles, 64-366 lot 11) or with 75 µl of non-immune rabbit serum for 18 h at 4 °C. Immune complexes in the samples treated with anti-κ serum formed visible precipitates after centrifugation in a Beckman Microfuge. The supernatants were aspirated, diluted a further 1:2 in normal mouse serum (final dilution 1:4) and then tested for an inhibitory effect on <sup>125</sup>I-insulin binding, according to the protocol described in Table 1. Results are the means of two experiments each performed in triplicate.

membranes with dilutions of NZO serum resulted in inhibition of subsequent <sup>125</sup>I-insulin binding (Table 1). For unknown reasons, undiluted serum either had no effect or actually increased binding slightly. Inhibition was specific for <sup>125</sup>I-insulin and was not seen for <sup>125</sup>I-growth hormone. However, preliminary experiments have shown a smaller effect of NZO serum on binding to NZO membranes, which may be due to antigenic modulation by previous exposure to antibody as the affinity of the receptor in NZO membranes is impaired (data not shown). Maximum inhibition of binding was 20-80% of control and was seen at dilutions between 1:4 and 1:32. Inhibition was never observed at serum dilutions greater than 1:64. Re-exposure of membranes to fresh serum did not cause a further decrease in binding (data not shown). Similar results were obtained in heterologous systems using either human placental membranes or cultured human IM-9 lymphocytes as receptor sources. Treatment of NZO sera with anti-mouse κ antiserum partially removed the inhibitory activity (Table 2), suggesting that the inhibitory factor was an immunoglobulin.

An immunoprecipitation assay for receptor antibodies was performed by incubating serum with Triton-solubilised mouse liver or human placental membranes pre-labelled with <sup>125</sup>I-insulin, followed by addition of a second (precipitating) antibody<sup>20,21</sup>. Solubilised mouse liver receptors were immunoprecipitated by NZO serum in a dose-dependent manner, after addition of anti-mouse globulin antiserum (Table 3). Precipitation of <sup>125</sup>I-insulin radioactivity in excess of control was not observed with NZO sera in the absence of receptor, thereby excluding the possibility of antibodies to insulin itself.

**Table 3** Immunoprecipitation of <sup>125</sup>I-insulin-labelled receptors by NZO serum and anti-mouse globulin antiserum

	Control serum	NZO serum			
		1:10 <sup>3</sup>	1:10 <sup>2</sup>	1:20	1:10
c.p.m. (% of total)	1.2	1.2	1.7	3.5	5.9

Normal mouse liver membranes (~50 mg ml<sup>-1</sup>) were solubilised by mixing them with 1% Triton (v/v)-0.1 M sodium phosphate buffer, pH 7.4, for 1 h at 24 °C. The mixture was then centrifuged at 200,000g for 1 h. 20 µl (~80 µg of protein) of the clear supernatant containing solubilised insulin receptors was incubated for 1 h at 24 °C with a trace amount of <sup>125</sup>I-insulin (50 pg; ~13,000 c.p.m.) in a total volume of 180 µl of 0.1 M sodium phosphate buffer. Pooled NZO serum (20 µl) serially diluted in control NZW serum, or control serum alone, was then added and incubations continued for a further hour at 24 °C. The mixtures were cooled to 4 °C and 50 µl of rabbit anti-mouse globulins (Behring Diagnostics lot A2929C) was added for a further 4 h at 4 °C. Immune complexes, which formed a visible suspension, were precipitated by centrifugation in a Beckman microfuge, washed once with 0.1% Triton buffer and counted in a gamma counter. When the receptor was omitted from the initial Triton buffer-<sup>125</sup>I-insulin mixture, radioactivity subsequently present in the immune precipitate was equivalent to that in the control, thus excluding the presence of antibodies to <sup>125</sup>I-insulin itself. Results are the means of two experiments each performed in triplicate.

Immunoprecipitation was more sensitive than the binding inhibition assay, activity being detected up to serum dilutions of 1:100 with titres (serum dilutions causing half-maximum immunoprecipitation) ranging from 1:10 to 1:40. We attempted to determine the class of antibody involved by using a variety of second antisera to mouse immunoglobulins A, G and M. Antiserum to mouse IgA was ineffective. Weak immunoprecipitation was obtained in the majority of sera tested by using either anti-mouse 7S globulins or anti-mouse IgM. We are investigating the possibility that the receptor antibody in NZO sera may not be well recognised by commercially available antisera, or may be a low molecular weight IgM.

A number of lines of evidence favour an immune basis for the metabolic abnormalities in NZO mice. First, the mice are distantly related to the autoimmune NZB strain<sup>1,2,15</sup>. Second, the Bielschowskys<sup>2</sup> demonstrated improvement of obesity, hyper-

glycaemia and insulin resistance during pregnancy and after treatment with stilboestrol. Although pregnancy is a state of 'physiological' insulin resistance it is also a state of 'immunological inertia'<sup>22</sup>, which might explain its beneficial effect in an autoimmune disease. Remission after stilboestrol therapy may have been due to the immunosuppressive effect of the steroid, particularly as gonadectomy was without effect in either sex<sup>2</sup>. Finally, the finding in NZO mice of a relatively high incidence of spontaneous and carcinogen-induced tumours<sup>23</sup> would be consistent with an underlying immune disorder.

The relationship of the antibody to age, sex and metabolic status is now being studied to define the pathological role of the antibody and to determine whether the NZO model is the counterpart of the recently described human syndrome of insulin-resistant diabetes associated with antibodies to the insulin receptor<sup>19,21</sup>.

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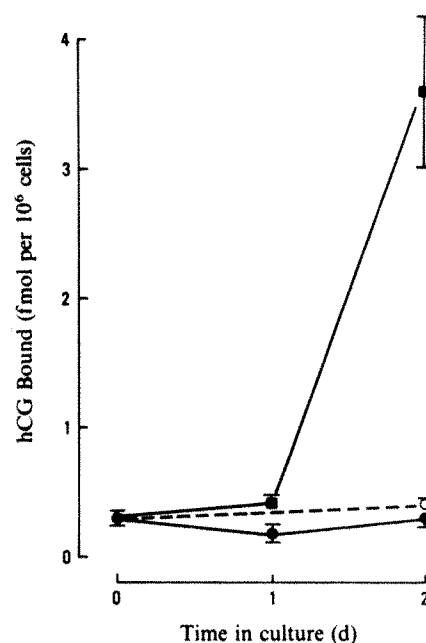
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## FSH induction of functional LH receptors in granulosa cells cultured in a chemically defined medium

THE rat ovarian granulosa cell is an excellent model for studying the mechanisms and control of hormone-dependent cell differentiation. During graafian follicle development, the granulosa cells sequentially develop specific membrane receptor sites for follicle-stimulating hormone (FSH)<sup>1,2</sup> and luteinising hormone (LH)<sup>3</sup>. *In vivo* studies on the mechanism of granulosa cell differentiation have established that FSH induces the



**Fig. 1** FSH induction of hCG binding sites in rat granulosa cells cultured in a chemically defined medium. ●, Without FSH (in the presence or absence of serum); ○, with FSH and 10% hypophysectomized female rat serum; ■, with FSH in the absence of serum. Immature female rats (Sprague-Dawley, 23–25 d old) were hypophysectomized and implanted with a 10-mm silastic capsule containing diethylstilboestrol (DES) to stimulate granulosa cell proliferation. Five days after hypophysectomy, the ovaries were removed and the granulosa cells collected and cultured as previously described<sup>9</sup>. The culture medium consisted of McCoy's 5a medium (modified; GIBCO) supplemented with 2 mM L-glutamine, 100 U ml<sup>-1</sup> penicillin and 100 µg ml<sup>-1</sup> streptomycin sulphate. The following agents were added where indicated; purified oFSH (Papkoff G4-150C; FSH potency = 50 × NIH-FSH-SI U per mg; LH potency < 0.01 NIH-LH-SI U per mg); androstenedione (10<sup>-7</sup> M; substrate for aromatization) and 10% animal serum from immature hypophysectomized female rats. The granulosa cells were cultured for 2 days in a 95% air–5% CO<sub>2</sub> incubator (37 °C). After the incubation, the granulosa cells were scraped from the dish with a rubber policeman, washed twice with 5-ml portions of Dulbecco's phosphate buffer with 0.1% bovine serum albumin (BSA) and the final pellet resuspended in the same buffer. Cell number was determined using a haemocytometer. Highly purified hCG (CR-119; 11,600 IU per mg) was iodinated by the procedure of Greenwood *et al.*<sup>10</sup>. The specific activity (80,000–130,000 c.p.m. per ng) and maximal binding capacity (40–45%), were determined by radioligand receptor assay using rat testicular membranes<sup>11</sup>. Samples (50 µl) of granulosa cells (1 × 10<sup>6</sup> cells) were incubated for 16 h (23 °C) with 100 µl of [<sup>125</sup>I]-hCG (~1 × 10<sup>-10</sup> M) with and without unlabelled hCG (100 IU Pregnyl)<sup>12</sup>. The absence of androstenedione from media did not modify the FSH effect, indicating that FSH alone induced the LH/hCG receptors. Data points indicate mean ± s.e. of six separate experiments with five dishes per determination per experiment.

appearance of the LH receptor sites in the granulosa cell<sup>4,5</sup>. The FSH-induced increase in LH receptors is essential for preparing the graafian follicle for the pre-ovulatory surge of LH which initiates ovulation and subsequent luteinisation of the granulosa cells. Studies of cultured granulosa cells have led to the suggestion that the FSH induction of LH receptors is not a direct process but requires an interaction between the granulosa cell and other ovarian cell types, a concept consistent with the known importance of cell–cell interaction in cell differentiation<sup>6</sup>. This hypothesis stemmed from the observation that LH receptors can be induced by FSH in granulosa cells *in vivo* and in organ cultures of intact ovarian follicles, but not in isolated granulosa cells cultured as monolayers in medium containing serum<sup>7,8</sup>. We show here that the inability of previous workers<sup>7,8</sup> to induce LH receptors in isolated granulosa cells may have been due to the use of serum in their tissue culture medium. We demonstrate that specific, high-affinity LH/hCG receptors can be induced by FSH in isolated granulosa cells cultured in a chemically defined medium, but not in isolated granulosa cells cultured with serum. In addition, we show that these receptors are capable of mediating important steroidogenic responses.

As shown in Fig. 1, the hCG-binding capacity of freshly collected granulosa cells was very low ( $0.32 \pm 0.014$  fmol per  $10^6$  cells;  $192 \pm 8$  sites per cell; mean  $\pm$  s.e. of six separate experiments). Culturing granulosa cells for 2 days in medium containing purified ovine FSH and 10% serum from hypophysectomised female rats (or serum from horse, pig or fetal calf), did not increase the  $^{125}$ I-hCG-binding capacity as compared with untreated control cultures or freshly isolated granulosa cells (Fig. 1). In contrast, culturing granulosa cells in serum-free medium containing FSH resulted in a dramatic increase in  $^{125}$ I-hCG binding as compared with control cells (Fig. 1). A small increase in binding was observed at day 1, after which the binding capacity increased 10-fold, to reach  $3.61 \pm 0.60$  fmol per  $10^6$  granulosa cells ( $2,095 \pm 325$  sites per cell) by day 2.

Figure 2a shows that the hCG receptors induced by FSH are hormone specific. Increasing concentrations of unlabelled hCG, but not prolactin, competed with  $^{125}$ I-hCG for binding sites in a dose-dependent manner. Moreover, unlabelled FSH inhibited binding only at doses 1,000 times higher than those of hCG, an inhibition that can be explained by contamination of the FSH preparation with LH. Scatchard analysis of the binding data (Fig. 2b) suggested the presence of two classes of  $^{125}$ I-hCG-binding sites. This is consistent with our previous studies using granulosa cells from adult rats<sup>12</sup>. The calculated  $K_d$  ( $1.4 \times 10^{-11}$  M) of the high-affinity binding site (Fig. 2b) is of the same order as that determined in previous studies with rat granulosa cells<sup>3,12</sup>. Furthermore, FSH treatment for 2 days *in vivo* increased  $^{125}$ I-hCG binding in the granulosa cell to a level comparable with that found in the present *in vitro* study (unpublished result).

To determine if the LH/hCG receptors induced by FSH were functionally active, we investigated the effects of purified LH and FSH on the biosynthesis of progesterone and oestrogen (Table 1). In this experiment, the granulosa cells were cultured for 2 days in a chemically defined medium with and without LH or FSH, washed, and then recultured for another 2 days in the presence and absence of the purified gonadotropins. During the 0–2 and 2–4 day culture period, FSH caused a marked stimulation of oestrogen and progesterone production, whereas LH had little effect, indicating that the granulosa cells are responsive to FSH, but not to LH. However, after 2 days of FSH priming LH caused a highly significant ( $P > 0.001$ ) increase in both oestrogen and progesterone production compared with the control (FSH; control) cultures. This result shows that the LH/hCG receptors induced by FSH are functionally active. The present finding that purified LH stimulates oestrogen formation is important as it demonstrates for the first time that LH, like FSH, can stimulate aromatase enzyme activity in the granulosa cell.

**Table 1** Induction by FSH of the ability of purified LH to stimulate progesterone and oestrogen production by isolated rat granulosa cells *in vitro*

Treatment 0–2 d; 2–4 d	Progesterone (ng ml <sup>-1</sup> )		Oestrogen (ng ml <sup>-1</sup> )	
	Day 0–2	Day 2–4	Day 0–2	Day 2–4
Control; Control	$0.10 \pm 0.01^*$	$0.22 \pm 0.01$	$< 0.04$	$< 0.04$
LH; LH	$0.50 \pm 0.01$	$0.27 \pm 0.01$	$0.19 \pm 0.01$	$0.07 \pm 0.01$
FSH; FSH	$21.67 \pm 1.92$	$8.35 \pm 0.71$	$4.28 \pm 0.22$	$4.09 \pm 0.22$
FSH; LH	—†	$7.32 \pm 0.47^\ddagger$	—	$2.49 \pm 0.14^\ddagger$
FSH; Control	—	$1.73 \pm 0.13$	—	$1.11 \pm 0.10$

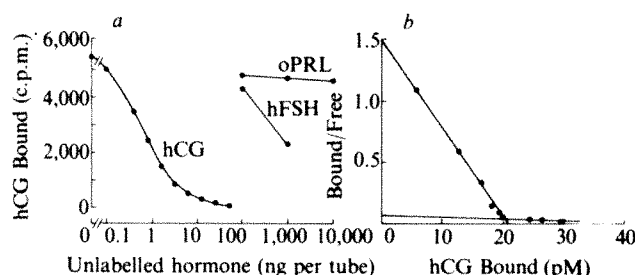
Granulosa cells ( $1 \times 10^5$  viable cells) were cultured for 4 days in McCoy's 5a medium with  $10^{-7}$  M androstenedione, either alone or with purified Papkoff ovine FSH ( $100$  ng ml<sup>-1</sup>) or purified oLH ( $100$  ng ml<sup>-1</sup>; Papkoff; G3-222B; LH potency =  $2.75$  NIH-LH-SI U per mg; FSH potency =  $0.001$  NIH-FSH-SI U per mg). After 2 days the media were collected, and the granulosa cells were washed twice with 2-ml portions of McCoy's 5a medium. The cells were then recultured for 2 days in 1 ml of fresh medium containing  $10^{-7}$  M androstenedione and hormones where indicated. Progesterone and oestrogen in the media were measured by radioimmunoassay<sup>13</sup>.

\* Mean  $\pm$  s.e.;  $n = 8$ .

† Same values designated in the FSH (day 0–2).

‡ Significantly different from control group (FSH; control) ( $P < 0.001$ ) as determined by analysis of variance.

The present results demonstrate that purified FSH can induce the appearance of specific, high-affinity LH/hCG receptors in isolated granulosa cells and that the LH/hCG receptors are capable of mediating two important biological responses, namely, oestrogen and progesterone biosynthesis. This direct action of FSH in the granulosa cell can explain the increased responsiveness of the developing pre-ovulatory follicle to LH during the follicular phase of the reproductive cycle. The fact that the characteristics of the LH/hCG receptors ( $K_d$  and number of binding sites) induced in the granulosa cell by FSH *in vitro* are similar to those induced by FSH *in vivo*, suggested that the present *in vitro* observations reflect a normal physiological event. Our finding that FSH induces the appearance of LH/hCG receptors in isolated granulosa cells cultured in a chemically defined medium (without serum) strongly suggests that this basic action of FSH does not require the participation of other ovarian cells, as recently proposed by Nimrod *et al.*<sup>7</sup>.



**Fig. 2** Specificity and binding characteristics of LH/hCG receptors induced by FSH *in vitro*. *a*, Specificity of  $^{125}$ I-hCG binding to granulosa cells after 2 days of culture with oFSH ( $100$  ng ml<sup>-1</sup>) in chemically defined medium (without serum). Granulosa cells ( $1 \times 10^6$  cells) (prepared as described in legend to Fig. 1) were incubated in  $300$   $\mu$ l (phosphate buffered saline– $0.1\%$  BSA) buffer for  $16$  h ( $23^\circ\text{C}$ ) with  $20,000$  c.p.m. of  $^{125}$ I-hCG and the indicated amounts of unlabelled hCG (CR-119), purified human FSH (NIH-FSH-HSI) or ovine prolactin (NIH-P-SI2). Data points represent the mean of four determinations. *b*, Scatchard analysis of binding data from displacement curve shown in *a*.  $K_d$ , Dissociation constant =  $1.4 \times 10^{-11}$  M; number of binding sites =  $3,157$  sites per cell.

Our failure to induce LH/hCG receptors with FSH in granulosa cells cultured in medium containing serum is consistent with previously published data<sup>7,8,14</sup>. Moreover, our results are consistent with those of an earlier study<sup>14</sup> which reported an FSH stimulation of LH binding in cultured porcine granulosa cells, although the data from these cells were variable and complicated by the loss of pre-existing LH receptors during the incubation. The nature of the inhibitory effect of serum is unknown; however, it may result from the presence of factors in serum which have been reported to inhibit FSH binding to receptors<sup>15</sup>.

Experiments with animal cells in culture have usually used serum because the maintenance and growth of functionally differentiated cell cultures require certain factors found in serum<sup>16</sup>. However, the addition of serum to culture media has made it difficult, sometimes impossible, to interpret the action of specific regulatory substances. We conclude that the *in vitro* culture system described here offers an important model for investigating and defining the control mechanisms of hormone-dependent differentiation of a normal mammalian cell in a chemically defined medium.

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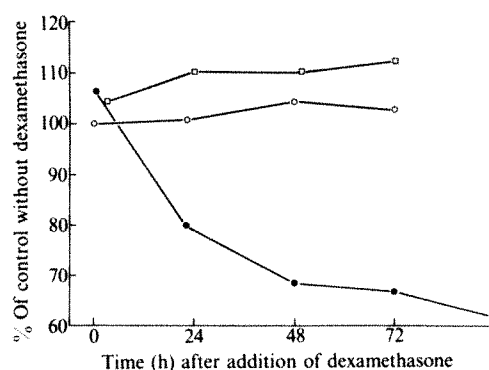
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## Glucocorticoids inhibit expression of Fc receptors on the human granulocytic cell line HL-60

GLUCOCORTICOIDS decrease levels of circulating peripheral lymphocytes and immunoglobulins, inhibit mitogen- and antigen-induced blastogenesis of cultured lymphocytes and decrease bactericidal activity of granulocytes and other phagocytic cells<sup>1-4</sup>. Fc receptors on granulocytes are thought to play a vital part in adherence of antibody-coated particles to the surfaces of these cells, a step which initiates phagocytosis<sup>5</sup>. Factors which regulate the expression of the Fc receptor could therefore influence phagocytic capability, and it has been suggested that glucocorticoids might act in such a way<sup>6,7</sup>. We report here that glucocorticoids inhibit expression of the Fc receptor on the human promyelocytic cell line HL-60 (ref. 8). This inhibition is not accompanied by increased cell death, reduced proliferation or a general reduction in protein synthesis.

For assay of Fc receptors, IgG1 was purified from the serum of a patient with multiple myeloma. The precipitate obtained by 45% saturation with ammonium sulphate was dissolved in acetic acid-ethylene diamine buffer (0.1M, pH 7.0) and desalted by passing through a column (1×20 cm) of Biogel P-100 (Bio-Rad). The excluded peak from the column was placed on a column (1×15 cm) of Sephadex QAE-50 (Pharmacia) and eluted with acetic acid-ethylene diamine buffer. The IgG1 obtained was pure by immunoelectrophoresis, and contained only traces of other proteins by gradient gel and cellulose acetate electrophoresis. The protein was iodinated with <sup>125</sup>I (Amersham, 11-17 Ci μg<sup>-1</sup>) by the solid phase lactoperoxidase method<sup>9,10</sup>. Protein concentration was determined by absorbance at 280 nm ( $A_{280}^{1\%} = 11.4$ ). Specific activities were between 20 and 57 Ci mmol<sup>-1</sup>.

This labelled IgG1 was used to determine the number and affinity of Fc binding sites by the modification of the competitive binding assay of Unkeless and Eisen<sup>11</sup> described in the legends to Figs 1 and 2. The assay showed high specificity for the Fc fragment of IgG1. In competition experiments with 0.5-5 nM



**Fig. 1** Time course with HL-60 cells of the effect of 200 nM dexamethasone on Fc receptor sites per cell (●), leucine incorporation (□) and viable cell counts (○). HL-60 cells\* were cultured in RPMI 1640 (GIBCO) with 10% fetal calf serum, penicillin (50 units ml<sup>-1</sup>) and gentamycin (50 μg ml<sup>-1</sup>) at 37°C in humidified room air containing 5% CO<sub>2</sub>. Dexamethasone dissolved in medium at 0.1 mM was added at time zero to three flasks containing 50 ml cell suspension (2×10<sup>5</sup> cells per ml). An equivalent amount of medium was added to three control flasks. At 15 min and 24, 48 and 72 h, 4×10<sup>6</sup> cells were removed and washed twice with 15 ml Dulbecco's phosphate-buffered saline (GIBCO) containing 1 mg ml<sup>-1</sup> bovine serum albumin (2×recrystallised, Calbiochem) (PBS-BSA) at room temperature. The cells were incubated for 30 min in 15 ml PBS-BSA (2-8×10<sup>5</sup> cells per ml) at 37°C with 60 c.p.m. shaking to dissociate endogenous immunoglobulins in fetal calf serum from the Fc receptors. They were then resuspended in PBS-BSA at 5×10<sup>7</sup> cells per ml, and 20 μl aliquots were incubated in duplicate with 20 μl of <sup>125</sup>I-labelled IgG1 (40 nM), with and without unlabelled IgG1 (1 μM) in albuminised 1.5-ml conical polypropylene Eppendorf centrifuge tubes (Brinkman) for 30 min at 37°C with 60 c.p.m. shaking. At the end of the incubation the cell suspension was pipetted over 350 μl fetal calf serum at 0°C in 400-μl microfuge tubes (Brinkman) and centrifuged for 30 s at 10,000g. The supernatant was rapidly aspirated and the tips of the tubes containing the cell pellet counted in a gamma counter at an efficiency of 70% for <sup>125</sup>I. The difference between the c.p.m. bound in the presence (an almost negligible amount, as shown in Fig. 2) and absence of 1 μM competing unlabelled IgG1 (that is, the c.p.m. corresponding to saturably bound IgG1) was calculated as molecules per cell. Since at 40 nM IgG1 nearly saturates the Fc receptor sites (see Fig. 2) this value gives a close estimate of the total number of Fc receptor sites per cell. Leucine incorporation was measured by removing 100-μl aliquots from each of the flasks and incubating for 4 h at 37°C under 5% CO<sub>2</sub> in humidified room air in microtitre plates (Costar) with 100 μl of a solution (3 μCi ml<sup>-1</sup>) of <sup>3</sup>H-leucine (NEN, 297 Ci mol<sup>-1</sup>) in GIBCO minimum essential medium. Cells were collected on glass fibre filters using a multiple automated sample harvester, and after drying were counted in a toluene-based scintillation fluid at an efficiency of 50% for <sup>3</sup>H. Viable cell counts were determined as the product of the fraction viable by Trypan blue exclusion and the cell count determined with a Coulter counter. Viabilities of control and dexamethasone-treated cells were not significantly different, and always remained above 95%. Each point represents the mean for determinations on three control flasks and three flasks with dexamethasone. For the controls, the number of Fc receptor sites remained essentially constant at about 16,000 sites per cell over the whole time period; the rates of leucine incorporation increased slightly, and the viable cell counts increased from 200 to 650 cells per μl.

<sup>125</sup>I-labelled IgG1 the Fc fragment, prepared by papain hydrolysis and affinity chromatography, at 10 nM reduced binding of labelled IgG1 by 50% and at 100 nM by 90%, as effectively as unlabelled IgG1. The Fab fragment, similarly prepared, did not compete at all. Human IgG3 competed as well as IgG1, whereas IgG2 and IgG4 competed weakly and IgA did not compete. Protein A (Pharmacia) at 100 nM reduced binding by 50%. It failed to reduce binding further at concentrations as high as 50 μM, suggesting among other possibilities that there may be more than one class of Fc receptor on HL-60 cells.

Figure 1 illustrates the time course of effects of 200 nM dexamethasone on HL-60 cells. Within 24 h there was a significant decrease in the number of Fc receptor sites, which by 48-72 h had fallen to ~65% of the controls without steroid. In other experiments the levels reached varied from 75 to 40%. They remained low for at least 8 d in the continued presence of dexamethasone, but returned to normal within 48 h of removal of the steroid. Over the 72-h period there was no decrease in overall leucine incorporation, so there was no general reduction of protein synthesis. Similarly, cell proliferation was not decreased.



The reduction in the number of Fc receptor sites was not associated with a change in the affinity of the receptor for IgG1. Control cells and cells treated with 1  $\mu$ M dexamethasone for 72 h bound IgG1 with the same dissociation constant of 10 nM (Fig. 2).

Prednisolone and cortisol at 1  $\mu$ M after 48 h reduced Fc receptor sites from 14,400 to about 8,000 per cell. Oestradiol and progesterone at 1  $\mu$ M had no effect. Dexamethasone gave a half-maximal response at 10 nM, comparable to effects of dexamethasone on other cellular processes<sup>12</sup>. This concentration is consistent with a process mediated by binding to glucocorticoid receptors in these cells, since it corresponds with the dissociation constant we have determined separately for binding of dexamethasone to glucocorticoid receptors in HL-60 cells at 37 °C. These cells contain numbers of glucocorticoid receptor sites similar to myeloblasts from patients with acute myelogenous leukaemia<sup>13</sup>. After Fc receptors are reduced to about 50% by treatment with trypsin, their reappearance is almost completely blocked by glucocorticoids. In the absence of steroid they return to normal after 48 h.

In two previous studies<sup>4,14</sup>, effects of glucocorticoids on Fc receptors in human monocytes were investigated. The only effect detected, a decrease in IgG binding, required steroid concentrations of 0.1–1 mM, far above pharmacological concentrations, and the effect was not specific for glucocorticoids<sup>14</sup>. The duration of exposure to steroids, 90–120 min, was much less than we find necessary to produce significant effects. Furthermore, the immune adherence techniques used would probably not detect effects of the kind we describe. Methods involving rosette formation with antibody-coated red cells or binding of fluorescence-labelled immune complexes can detect cells with more than some undetermined threshold level of Fc receptors (probably 10,000–20,000 sites per cell), but do not permit accurate quantitation of binding.

Our finding that glucocorticoids reduce the number of Fc receptors on HL-60 cells is important in at least two ways. First, it establishes in a pure cell line an effect that is manifested on a discrete membrane structure and that, in contrast to what is often observed<sup>12</sup>, is not accompanied by general inhibition of protein synthesis. Second, it may provide a molecular explanation for the suppressive effects of glucocorticoids on the phagocytosis and destruction of autologous tissue that characterise

some immune responses mediated by Fc receptors. The observation<sup>16,17</sup> that patients with diseases such as autoimmune haemolytic anaemia and autoimmune thrombocytopenia frequently show clear improvement and decreased phagocytic activity in their granulocytes after treatment for 24–48 h with glucocorticoids could well be explained by a decrease in the number of Fc receptors. The time course is too rapid to be accounted for by known effects on human immunoglobulin levels<sup>2</sup> but is similar to that needed for the reduction in Fc receptors that we describe here. The assay for Fc receptors may also prove useful for studying other factors which regulate this immunologically important structure.

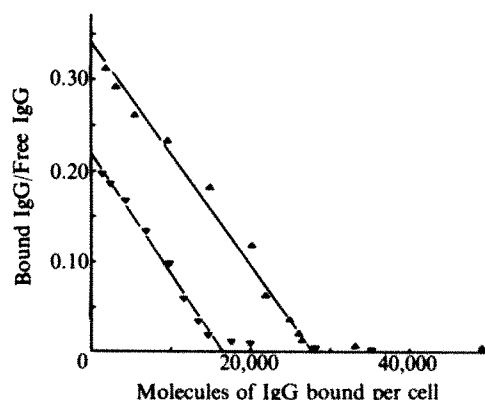
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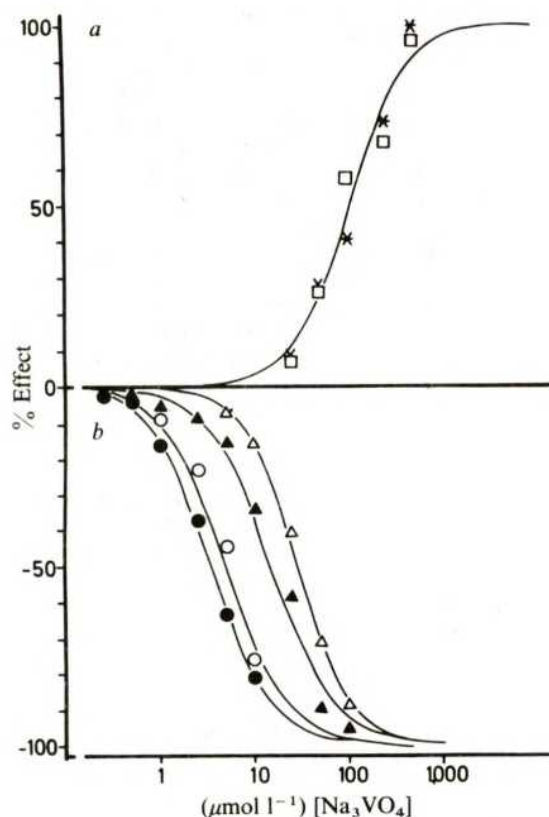


**Fig. 2** Scatchard plot of binding of IgG1 to Fc receptors of HL-60 cells after incubation with 1  $\mu$ M dexamethasone.  $\nabla$ , Controls without dexamethasone.  $\Delta$ , Cells incubated with 1  $\mu$ M dexamethasone. Cells were incubated with or without dexamethasone for 96 h and washed in PBS-BSA as described for Fig. 1. They were then resuspended at  $1.6 \times 10^8$  cells per ml in PBS-BSA. Aliquots were incubated as described for Fig. 1 with  $^{125}$ I-labelled IgG1 at 12 concentrations from 100 pM to 1  $\mu$ M. After the incubation the tubes were centrifuged at 10,000g for 10 s and 20  $\mu$ l supernatant removed and counted to determine the final concentration of free labelled IgG1. The tubes were then cooled to 3°C, 1.2 ml of PBS-BSA at 0 °C was added and the cells were resuspended. After 5 min the tubes were centrifuged at 12,000g for 4 min, the supernatant aspirated and the tips counted as described for Fig. 1 to determine bound  $^{125}$ I-labelled IgG1. The data are plotted by the method of Scatchard<sup>15</sup>. Nonsaturably bound IgG1 was not subtracted for these results. Bound IgG and free IgG on the ordinate are given in units of c.p.m. per 20  $\mu$ l of cell suspension; the values on the ordinate therefore represent the bound IgG1 as a fraction of the free IgG1 at equilibrium.

## Negative and positive inotropic action of vanadate on atrial and ventricular myocardium

VANADATE (vanadium in the +5 oxidation state) occurs widely in animal tissues<sup>1</sup>, and has been suggested to be the first regulatory agent of  $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ ; it inhibits ATPase preparations from kidney and red blood cells at very low concentrations<sup>2–4</sup>. Hackbarth *et al.* have shown that vanadate produces positive inotropic effects in isolated cat papillary muscles<sup>5</sup>, raising the question of whether this positive inotropic effect is accompanied by an inhibition of myocardial  $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ , and, if so, whether both effects occur at similar concentrations of vanadate. We report here that we have observed a striking difference in the inotropic actions of vanadate on isolated heart preparations. Although the force of contraction was reduced in atria, it was increased in ventricular myocardium. This dissociation correlated well with changes in transmembrane potential but not with inhibition of  $(\text{Na}^+ + \text{K}^+)\text{ATPase}$  isolated from both atrial and ventricular myocardium.

Left and right atria and papillary muscles from guinea pig and cat, and atrial and ventricular trabeculae of the cow, were mounted in glass chambers for measuring isometric contraction. The bathing solution (20 ml), containing (mmol l<sup>-1</sup>) NaCl 118, KCl 4.7, CaCl<sub>2</sub> 2.5, MgSO<sub>4</sub> 1.6, NaHCO<sub>3</sub> 24.9, NaH<sub>2</sub>PO<sub>4</sub> 1.2



**Fig. 1** Change in the force of contraction in papillary muscle (*a*) and atria (*b*).  $ED_{50}$  values ( $\mu\text{mol l}^{-1} \pm \text{s.e.m.}$ ) were calculated by fitting the logit function to the experimental data using the least-square method: papillary muscle, guinea pig ( $\square$ )  $110.3 \pm 1.9$ , cat ( $*$ )  $124.1 \pm 2.6$ ; left atria, guinea pig ( $\circ$ )  $4.73 \pm 0.10$ , cat ( $\triangle$ )  $28.1 \pm 0.47$ ; right atria, guinea pig ( $\bullet$ )  $3.29 \pm 0.06$ , cat ( $\blacktriangle$ )  $14.8 \pm 0.28$ . The curves were plotted according to the equation

$$y(x) = \frac{100}{1 + 10^{-s(x - \log ED_{50})}}$$

where  $s$  = slope and  $x$  = log concentration<sup>6</sup>.

and glucose 11.1, was equilibrated with 95%  $\text{O}_2$  and 5%  $\text{CO}_2$  (pH 7.4) at 31 °C. Increasing doses of  $\text{Na}_3\text{VO}_4$  (Fluka) were added to the bathing solution cumulatively every 15 min. The preparations were driven electrically by platinum electrode stimulation at 1 Hz (guinea pig) or 0.5 Hz (cat, cow). Pulse duration was 1 ms (intensity 20% above threshold, preload of atria  $10^{-2}$  N, that of papillary muscles and trabeculae  $7 \times 10^{-3}$  N  $\text{mm}^{-2}$ ). Action potentials of the isometrically contracting atrial and ventricular tissues were recorded with glass micro-electrodes filled with 3 mol  $\text{l}^{-1}$  KCl (resistance 10–30 M $\Omega$ ) using a horizontal Perspex chamber, perfused with increasing concentrations of  $\text{Na}_3\text{VO}_4$ .

Figure 1 shows the inotropic action of vanadate. In a concentration range of 25–500  $\mu\text{mol l}^{-1}$ , vanadate increased the force of contraction of papillary muscles. In contrast, it strongly decreased the force of contraction of stimulated left atria and spontaneously beating right atria. The effect appeared immediately on perfusion and steady-state conditions were reached within 10 min. On washing with drug-free bathing solution, the force of contraction of the atria increased above control levels and regained initial values after about 15 min. At high concentrations of vanadate ( $>1$  mmol  $\text{l}^{-1}$ ) and 1 Hz stimulation frequency, a small increase in basal tension of guinea pig papillary muscles was observed, but without arrhythmic activity.

Figure 2 shows the corresponding action potentials. In guinea pig papillary muscles 500  $\mu\text{mol l}^{-1}$  vanadate broadened the action potential by about 15% at both 30 and 90% repolarisation, whereas in left atria 50  $\mu\text{mol l}^{-1}$  vanadate produced a shortening of the action potential by 60 and 70% at 30 and 90%

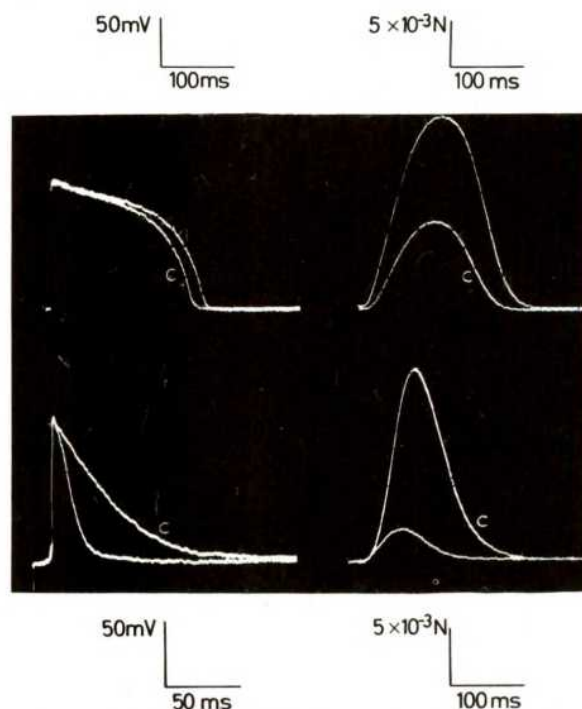
**Table 1** Enzyme activities of bovine and guinea pig heart preparations and the inhibitory effect of orthovanadate on the  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase

Beef	Activity	Atria		Ventricle	
		$\text{Mg}^{2+}$ -ATPase	$(\text{Na}^+ + \text{K}^+)\text{ATPase}$	$\text{Mg}^{2+}$ -ATPase	$(\text{Na}^+ + \text{K}^+)\text{ATPase}$
	$\text{ID}_{50}$	$\mu\text{M Na}_3\text{VO}_4$			
			$0.59 \pm 0.14$	$0.60 \pm 0.14$	
Guinea pig	Activity	Atria		Ventricle	
		$\text{Mg}^{2+}$ -ATPase	$(\text{Na}^+ + \text{K}^+)\text{ATPase}$	$\text{Mg}^{2+}$ -ATPase	$(\text{Na}^+ + \text{K}^+)\text{ATPase}$
	$\text{ID}_{50}$	$\mu\text{M Na}_3\text{VO}_4$			
			$0.75^*$	$0.62 \pm 0.13$	

Membrane ATPase preparations were derived using the method of Akera *et al.*<sup>7</sup>. Enzyme activities were determined with a coupled spectrophotometric assay<sup>8</sup>, in the presence of 50–100  $\mu\text{g}$  enzyme protein and (mmol  $\text{l}^{-1}$ )  $\text{Na}^+$  100,  $\text{K}^+$  10,  $\text{Mg}^{2+}$  5 and ATP 3, at 37 °C, pH 7.4, and were measured in  $\mu\text{mol PO}_4$  per mg protein per h. The  $\text{Mg}^{2+}$ -ATPase activity was measured in the presence of 1 mmol  $\text{l}^{-1}$  ouabain. Each activity is the result of four separate preparations determined in triplicate and each  $\text{ID}_{50}$  value the result of four determinations (with exception\*, where  $n = 1$ ) prepared from 48 guinea pigs. Values are means  $\pm$  s.e.m.

repolarisation, respectively. The upstroke velocity of the action potential of both atria and papillary muscles remained nearly unchanged. Similar results were obtained from atrial and ventricular preparations of cat and bovine heart. The time course of the vanadate effect on transmembrane potentials correlated with that of the change in inotropy.

The specific activities of the  $\text{Mg}^{2+}$ - or  $\text{Na}^+ + \text{K}^+$ -activated ATPase for bovine atrial and ventricular tissues are shown in Table 1. Despite the difference in enzyme activities, concentration–response curves for the action of sodium orthovanadate gave similar  $\text{ID}_{50}$  values for the inhibition of bovine atrial and ventricular  $\text{Na}^+ + \text{K}^+$ -activated ATPase. Guinea pig ventricular and atrial ( $\text{Na}^+ + \text{K}^+$ )ATPase was inhibited to a similar extent,



**Fig. 2** Action potentials from guinea pig papillary muscle (left upper trace) and from the left atrium (left lower trace) in control conditions (C) and under  $\text{Na}_3\text{VO}_4$  (14 min 500  $\mu\text{mol l}^{-1}$  for papillary muscle and 8 min 50  $\mu\text{mol l}^{-1}$  for the atrium). The related force of contraction in control and test conditions is shown in the right traces. The upstroke velocities of the action potentials were not significantly influenced by vanadate, being 150  $\text{Vs}^{-1}$  for the papillary muscle and 197  $\text{Vs}^{-1}$  for the atrium.

although the atrial enzyme activity was too low to allow more than one estimation in a preparation using 96 atria.

The positive and negative inotropic actions of vanadate are accompanied by a similar inhibition of the  $(\text{Na}^+ + \text{K}^+)\text{ATPase}$  of ventricular and atrial preparations, despite a higher activity of the  $(\text{Na}^+ + \text{K}^+)\text{ATPase}$  from ventricular myocardium. Negative inotropic changes occur at concentrations ( $1\text{--}100\ \mu\text{mol l}^{-1}$ ) which induce a 55–95% inhibition of bovine cardiac  $(\text{Na}^+ + \text{K}^+)\text{ATPase}$  preparations and a 15–60% inhibition of rubidium uptake in red blood cells<sup>4</sup>, whereas positive inotropic changes ( $50\text{--}500\ \mu\text{mol l}^{-1}$ ) correspond to a 90–100% inhibition of  $(\text{Na}^+ + \text{K}^+)\text{ATPase}$  preparations and a 55–80% inhibition of rubidium uptake<sup>4</sup>. It remains to be explained why high concentrations of vanadate ( $>1\ \text{mmol l}^{-1}$ ), which strongly inhibit  $(\text{Na}^+ + \text{K}^+)\text{ATPase}$  preparations, do not produce arrhythmias similar to those observed with high doses of cardiac glycosides.

Positive and negative inotropic effects correlate well with a broadening and shortening of the action potential, respectively. Therefore, we conclude that differing inotropic effects of vanadate are mainly due to the alteration of the transmembrane potential, but not primarily to the inhibition of cardiac  $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ . Another striking property of vanadate is its concentration-dependent stimulatory effect on the adenylate cyclase of rat fat cell membranes<sup>9</sup>. It is still possible that the positive inotropic action of vanadate on ventricular myocardium is caused by an increase in intracellular cyclic AMP. However, the negative inotropic effect of vanadate in atria which occurs at lower concentrations seems to be due to a shortening of the action potential.

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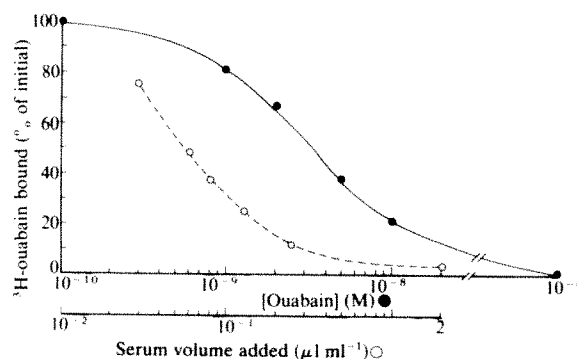
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## Endogenous digitalis-like activity in the plasma of the toad *Bufo marinus*

CARDIAC GLYCOSIDES derived from plants have a major role in the pharmacotherapy of cardiac disease, and, because they bind to and inhibit  $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ , are important in studies of ion transport mechanisms. In the animal kingdom, structurally related compounds with cardiotonic activity have been shown to exist in the poison glands of bufonid toads<sup>1</sup>, but this area has received little attention. Recently we developed a sensitive radioreceptor assay for the detection of endogenous 'digitalis-like' agonists in animals<sup>2</sup>. This assay depends on the ability of a substance to compete with  $^3\text{H}$ -ouabain for binding to  $(\text{Na}^+ + \text{K}^+)\text{ATPase}$  on human red cells. Using this assay, together with assays for inhibition of K transport and  $(\text{Na}^+ + \text{K}^+)\text{ATPase}$  activity, we found high concentrations of 'digitalis-like' activity in the skin of several species of Amphibia<sup>2</sup>. Although this activity is presumably present in skin as a protective toxin, it is possible that it might, under some circumstances,



**Fig. 1** Effect of ouabain (●) or serum (○) on the binding of  $^3\text{H}$ -ouabain to human RBC was assessed by a modification of the method of Gardner and Conlon<sup>4</sup>. Heparinised blood was washed 4 times with 140 mM choline chloride at 4 °C, the buffy coat was removed and RBC were resuspended at a haematocrit of 4% in an assay buffer of 140 mM NaCl, 30 mM HEPES and 10 mM glucose, pH 7.4. To 900  $\mu\text{l}$  of this RBC suspension was added 100  $\mu\text{l}$  of various dilutions of ouabain or serum and these mixtures were incubated at 37 °C for 1 h. Cells were then sedimented at 1,500 r.p.m., followed by resuspension in 225  $\mu\text{l}$  of the fresh assay buffer containing  $5 \times 10^{-10}\ \text{M}$   $^3\text{H}$ -ouabain (8 Ci mmol<sup>-1</sup>, NEN). After incubation at 37 °C for 70 min, bound and free ouabain were separated by washing 4 times in microfuge tubes with 140 mM choline chloride, followed by addition of 10% perchloric acid to the pellet and inversion of the tube into a scintillation vial containing Aquafuor (NEN). Each assay contained duplicate tubes in which  $^3\text{H}$ -ouabain binding was carried out in the presence of  $10^{-4}\ \text{M}$  unlabelled ouabain. This was taken to represent nonspecific binding, and was always less than 10% of total  $^3\text{H}$ -ouabain bound. The per cent of  $^3\text{H}$ -ouabain specifically bound to each group of cells was determined by subtracting the per cent bound to cells in the presence of  $10^{-4}\ \text{M}$  ouabain from the per cent actually bound in each experimental tube. This specific binding for each tube was divided by the specific binding to cells which were never exposed to serum or unlabelled ouabain, and this value was multiplied by 100, to give the value plotted on the vertical axis. The points which are plotted represent the mean of duplicates which varied less than 5%. The ouabain concentration on the abscissa represents the concentration in 1.0 ml of preincubation mix.

also function as an endogenous inhibitor of  $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ . We now demonstrate, using both receptor and immunoassays, that high concentrations of digitalis-like activity are present in the plasma of the toad, *Bufo marinus*. As cellular  $(\text{Na}^+ + \text{K}^+)\text{ATPase}$  would presumably be exposed to this endogenous digitalis-like activity, it may have an important, but as yet undetermined, function in this species.

Pre-exposure of human red blood cells (RBC) to dilutions of *Bufo marinus* serum followed by washing and resuspension in fresh buffer, caused a dose-dependent inhibition of subsequent  $^3\text{H}$ -ouabain binding to these cells. The dilution curve produced by serum was parallel to that produced by unlabelled ouabain (Fig. 1), 50% inhibition of  $^3\text{H}$ -ouabain binding being caused by a 1/20,000 dilution of serum and 95% inhibition being caused by a 1/1,000 dilution. Assuming that the activity has a binding affinity for human RBC  $(\text{Na}^+ + \text{K}^+)\text{ATPase}$  that is similar to that of ouabain, the apparent serum concentration in five toads ranges from  $2$  to  $5 \times 10^{-5}\ \text{M}$ . *Rana pipiens*, whose skin has little or no detectable activity (J. S. F. and Daly, in preparation), also had no serum activity detectable, even when tested at 1/10 dilution (data not shown). The digitalis-like activity was unaffected by heating to 100 °C for 15 min, but 95% of the activity was lost after dialysis for 48 h.

To characterise the ouabain binding sites on the  $(\text{Na}^+ + \text{K}^+)\text{ATPase}$  of bufonid RBC, we washed cells six times in 15 volumes of buffer to remove circulating digitalis-like activity, and then performed a  $^3\text{H}$ -ouabain binding assay as with human RBC. No specific  $^3\text{H}$ -ouabain binding was detectable over a range of tracer concentrations from  $5 \times 10^{-10}$  to  $5 \times 10^{-8}\ \text{M}$ , whereas binding was easily detectable in the same number of human RBC (data not shown).

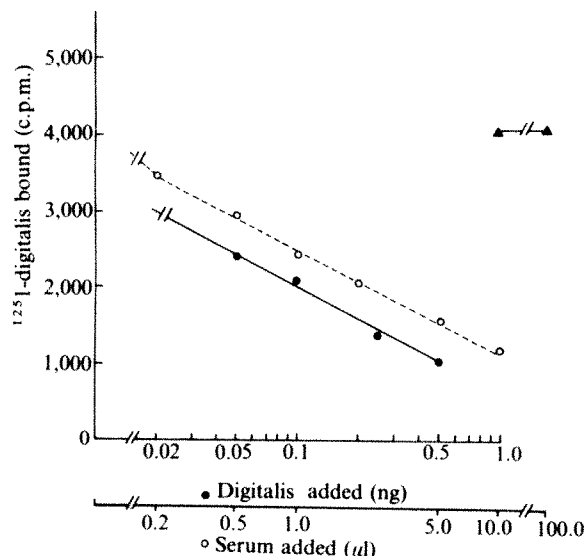


Serum from *Bufo marinus* cross-reacted strongly in a digitalis immunoassay. Serial dilutions produced displacement nearly parallel to that produced by digitalis itself (Fig. 2). In contrast, human serum produced no displacement. The data indicate that 1  $\mu$ l of bufonid serum contains 0.05 ng of immunoreactive digitalis-like activity.

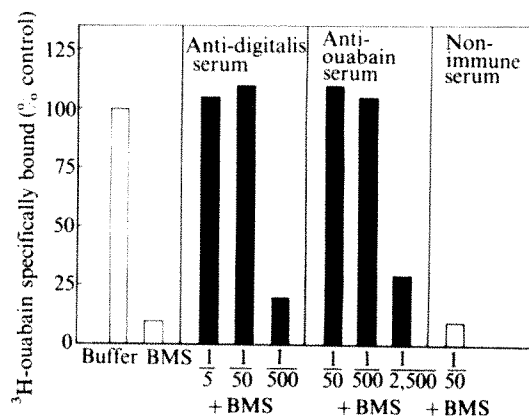
Preincubation of bufonid serum with antibodies to either digitalis or ouabain blocks the receptor competition (Fig. 3). Thus the  $^3$ H-ouabain binding-inhibitory activity seems to be caused by the same molecular species that cross-reacts with antibodies to digitalis and ouabain. This immunological evidence represents entirely independent confirmation that bufonid serum contains high concentrations of a digitalis-like activity.

These data suggest that the concentration of serum digitalis-like immunoactivity is  $\sim 10^{-7}$  M. When compared to the concentration as estimated by competition for receptor binding  $2-5 \times 10^{-5}$  M, it seems that the receptor binding activity of this molecule has been better conserved than has its cross reactivity with anti-digitalis and anti-ouabain antibodies. Direct structural studies will determine its precise relationship to the cardiac glycosides, and to known bufotoxins.

Several questions remain unanswered by these studies. First, although digitalis-like activity is present by both receptor-competition and immunological criteria, it remains to be shown that this serum activity is capable of inhibiting the activity of  $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ . Studies suggest that the serum activity is a fully potent inhibitor of the human enzyme (J. S. F. unpublished results). Second, it is not certain by what means *Bufo marinus* can survive with a serum concentration equivalent to more than 1,000 times the concentration of digitalis that produces toxicity



**Fig. 2** Dilution curves for digitalis (●) and bufonid serum (○) in a radioimmunoassay for digitalis. The immunoassay was carried out using a kit supplied by Corning Medical. Reagents include rabbit anti-digoxin antibody which is covalently linked to glass particles and suspended in phosphate-buffered saline with 1% bovine serum albumin, and  $^{125}\text{I}$ -digoxin tracer, comprising  $^{125}\text{I}$ -tyrosine linked to the digoxin molecule. The assay is carried out at room temperature in a total volume of 500  $\mu$ l. The indicated amounts of digitalis are added to the assay tubes in 100  $\mu$ l of pooled human serum to develop the dilution curve for digitalis. The indicated volumes of bufonid or human serum (▲) are similarly added to the assay tubes, with the difference between the indicated volume and 100  $\mu$ l being added as 140 mM NaCl. After incubation for 30 min at room temperature, glass beads were centrifuged at 1,600g for 10 min, supernatants decanted, and radioactivity in the pellets counted in a gamma counter. Points represent the mean of duplicates which varied less than 5%. Very similar results were obtained with serum from two other *Bufo marinus* toads. In this assay, digitoxin shows 3% cross-reactivity, and ouabain, testosterone, progesterone and spironolactone show negligible cross-reactivity.



**Fig. 3** Effect of anti-digitalis and anti-ouabain antisera on the ability of bufonid serum to inhibit  $^3\text{H}$ -ouabain binding to human RBC. *Bufo marinus* serum (BMS) was diluted 1/1,000 in an assay buffer of 140 mM NaCl, 30 mM HEPES and 10 mM glucose, pH 7.4. A 200  $\mu$ l aliquot of this BMS was incubated with 50  $\mu$ l of buffer, or with 50  $\mu$ l of sheep anti-digitalis serum, rabbit anti-ouabain serum or normal human serum which had been diluted in assay buffer to yield the final dilution indicated on the figure. A control tube comprised assay buffer alone. These tubes were incubated at 37  $^{\circ}\text{C}$  for 1 h, followed by the addition of 750  $\mu$ l of human RBC which had been washed 4 times in 140 mM choline chloride and then suspended in assay buffer, to give a final haematocrit of 5%. After an additional incubation for 1 h at 37  $^{\circ}\text{C}$ , cells were sedimented at 1,500 r.p.m., followed by resuspension in 225  $\mu$ l of fresh assay buffer containing  $5 \times 10^{-10}$  M  $^3\text{H}$ -ouabain (8 Ci mmol $^{-1}$ , NEN). After a final incubation at 37  $^{\circ}\text{C}$  (for 70 min, bound and free ouabain were separated by washing 4 times in microfuge tubes with 140 mM choline chloride, followed by addition of 10% perchloric acid to the pellet and inversion of the tube into a scintillation vial containing Aquaflo NEN). Each assay contained duplicate tubes in which  $^3\text{H}$ -ouabain binding was carried out in the presence of  $10^{-4}$  M unlabelled ouabain. This was taken to represent nonspecific binding, and was always less than 5% of total  $^3\text{H}$ -ouabain bound. The data shown are representative of two other experiments not described.

in man. *Bufo marinus* is known to be resistant to the effects of cardiac glycosides, requiring 100 times more ouabain to stop its heart *in vivo*, or to inhibit  $\text{Na}^+$  transport across its bladder *in vitro* than does *Rana pipiens*<sup>4</sup>. This relative resistance to ouabain is consistent with our observation that tracer  $^3\text{H}$ -ouabain binds poorly to bufonid cells. Recent experiments suggest that the bufonid enzyme binds ouabain with low affinity (J. S. F., unpublished results).

Although *Bufo* is clearly relatively resistant to the action of digitalis, high concentrations of this substance, in the range which we estimate (by receptor assay) to be present in its serum, inhibit short circuit current across the bladder of this species *in vitro*<sup>3</sup>. For this reason, it is possible that sufficient activity is bound to the enzyme at prevailing concentrations to tonically inhibit ATPase activity. Preliminary studies demonstrate that bladder from *Bufo marinus* contains easily detectable digitalis-like activity by receptor assay and immunoassay. Studies are in progress to determine what fraction of this extractable activity is actually bound to the bufonid  $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ . This should be of interest because the toad bladder is often used as a model system for  $\text{Na}^+$  transport in the distal nephron of the mammalian kidney. Perhaps it accounts in part for the observation that short circuit current across the toad bladder *in vitro* increases during equilibration of the bladder in buffer.

Thus we have demonstrated for the first time that a digitalis-like substance circulates naturally in the plasma of an animal. The effect of this circulating digitalis-like activity on the physiology of *Bufo marinus* is currently unclear, and the possibility that it tonically inhibits or regulates the  $(\text{Na}^+ + \text{K}^+)\text{ATPase}$  in this species needs further investigation.

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## Circular hydrogen bonds

CIRCULAR hydrogen bonds present a new, experimentally demonstrated principle showing how hydration water molecules and hydroxyl groups of macromolecules can cooperate to form a network-like pattern. Quantum chemical calculations show that chain-like H-bonds in the crystal lattice<sup>1</sup> are energetically favoured above individual ones<sup>2,3</sup>. This is due to the cooperative effect, which leads to increased H-bonding activity of an OH-group if it is already accepting or donating an H-bond. These linear structures can close up to form circular arrangements comprising four and more OH-groups<sup>4-6</sup>. Such circles have actually been described for crystal structures of ice<sup>7</sup> and of ice clathrates<sup>8</sup>, but in these cases the water molecules within the circles are related by crystallographic symmetry elements and are therefore not independent of each other. One should expect to find lattice-independent circular H-bonds in crystal structures of large O—H-rich molecules which co-crystallise with water of hydration, conditions which are satisfied by the cyclodextrin family. The smallest member,  $\alpha$ -cyclodextrin ( $\alpha$ -CD; cyclohexaamylose), consists of six  $\alpha$ (1,4)-linked glucose molecules and contains six primary and 12 secondary hydroxyl groups ((C<sub>6</sub>H<sub>10</sub>O<sub>5</sub>)<sub>6</sub>, molecular weight 973). From aqueous solution,  $\alpha$ -CD crystallises as hexahydrate,  $\alpha$ -CD·6H<sub>2</sub>O, and this complex, with a total of 120 hydroxyl groups (4 × 18 from  $\alpha$ -CD and 4 × 12 from the six H<sub>2</sub>O) in one unit cell has been studied by X-ray and neutron diffraction methods (ref. 9, and Klar, Hingerty and W.S., unpublished). Two other complexes, a second modification of the hexahydrate (K. Lindner and W.S., unpublished) and  $\alpha$ -CD·methanol·4H<sub>2</sub>O (ref. 10) have been investigated by X rays. As refinement in all cases is around  $R = 4\%$  for data extending to 0.89 Å resolution, hydrogen atom positions could be assigned. I discuss here results obtained from the X-ray/neutron study of  $\alpha$ -CD·6H<sub>2</sub>O.

Figure 1 shows a small, relevant section of the crystal structure of  $\alpha$ -CD·6H<sub>2</sub>O obtained from the X-ray/neutron study. Three circular H-bonded structures can be distinguished, one six-membered and two five-membered, all connected by water molecules W(1) and W(4). Two chain-type, linear H-bond structures extend on the left and right hand sides, linked with the circles by W(2) and W(4). Both chain structures with asymmetrical lengths of seven and eight O—H groups are formed by unidirectional O—H...O bonds which indicate the cooperative effect. As similar chains occur in small as well as in large molecule crystal structures<sup>1</sup>, they can be considered as general structural elements.

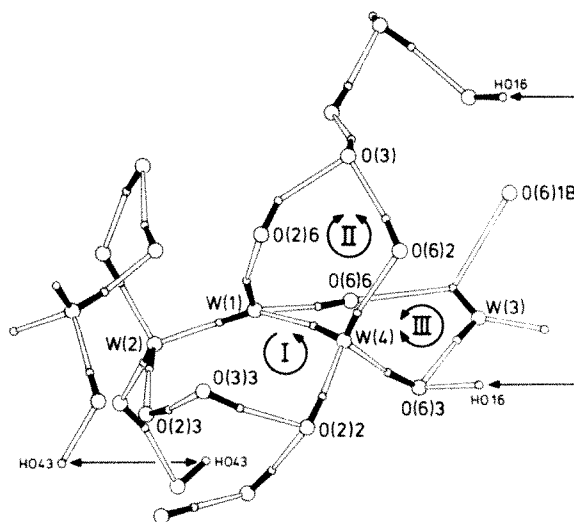
The three H-bonded circles shown in Fig. 1 all differ from each other. The six-membered circle I is formed by three interconnected water molecules W(1), W(2), W(4) and by three  $\alpha$ -CD hydroxyl groups of which O(2)2 and O(3)3 belong to the same molecule and O(2)3 is from a symmetry-related  $\alpha$ -CD. The six oxygen atoms form a distorted hexagon in an approximate boat shape, with W(1) and O(3)3 in bow/stern positions. In

this circle, H-bond cooperation is again effective because all O—H...O bonds run in the same direction. Therefore, this kind of circle is called *homodromic*.

The adjacent five-membered circles II and III comprise water molecules W(1) and W(4). Circle II contains three hydroxyl groups, of which two, O(2)6 and O(3)1, originate from one  $\alpha$ -CD molecule, and O(6)2 is from a symmetry-related molecule. W(4) acts as a double H-bond donor and thus generates two ...O—H...O—H chains which collide at the double acceptor O(3)1. Because this circle consists of two counter-running chains, it is called *antidromic* (circles with more randomly orientated chains would be called *heterodromic*). In circle III, in addition to water molecules W(1) and W(4), a third water, W(3), is enclosed and the two hydroxyl groups belong to two different  $\alpha$ -CD molecules. This circle is also *antidromic*, as W(3) is a double donor and W(1) a double acceptor for H-bonds. The H...O(6)6 distance, 2.72 Å is unusually long because this hydrogen is also bonded to O(6)1B at 2.85 Å and thus forms a bifurcated H-bond. Based on the sum of the van der Waals potential minimum radii, 1.50 Å for H and 1.65 Å for O, all H...O distances < 3.15 Å can be called bonding interactions and the H...O(6)6, O(6)1B distances are well within this range<sup>11</sup>.

It is remarkable that the three circles share the two water molecules W(1), W(4). The latter are not only used to fill empty gaps between the  $\alpha$ -CD molecules but they are also fully integrated into the H-bonding scheme. We can assume that the circular H-bonds are made possible through the water molecules which, because of their quadruple functionality and their low  $pK$  value ( $pK = 7$ ) relative to the  $\alpha$ -CD hydroxyl groups ( $pK \sim 12.3$  (refs 12,13)), can act as connectors and as shunts. The commonly observed tetrahedral arrangement of the H-bonded ligands is barely satisfied. Only W(2) and W(4) show approximate tetrahedral configuration (with O...W...O angles in the range 94° to 139°); the tetrahedron around W(1) is very distorted (angular range 66° to 142°) and the ligands around W(3) are in a planar-trigonal arrangement.

Quantum chemical studies on the three circles described here have shown that the total energy in each circle is about 2-4 kcal per circle smaller than the sum of the corresponding individual H-bonds (Lesyng and W.S., unpublished). The gain in energy on formation of circular H-bonds is thus similar to that calculated



**Fig. 1** A section of the  $\alpha$ -CD·6H<sub>2</sub>O crystal structure showing circular and chain-like H-bonds. The latter are marked by arrows, the former by roman numerals I-III. Circular arrows indicate the direction of the O—H...O hydrogen bonds. Circle I is homodromic and circles II, III are antidromic. Water and  $\alpha$ -CD hydroxyl oxygens are indicated by W and O, respectively; for further numbering see ref. 10. Ranges for O...O; H...O distances (Å) and O—H...O angles in H-bond circle I are 2.692-3.016 Å; 1.75-1.95 Å; 169-176°; in circle II, 2.747-3.021 Å; 1.86-2.14 Å; 150-171°, in circle III, 2.831-3.339 Å; 1.71-2.72 Å; 117-171°.

for chain-like H-bonds<sup>2,3</sup>, but the latter are more frequently encountered because the former are obviously restricted to large unit cells containing many hydroxyl groups and water molecules. Circular H-bonds might exist at the surfaces of large, hydrophilic molecules where, in combination with isolated and with chain-like H-bonds, they can link existing OH-groups or other hydrophilic residues using water molecules as connectors and shunts. It is likely that a pattern of circular and linear H-bonds can form, providing the basis for more extensive hydration shells. It is also tempting to regard the flickering cluster structure of bulk water<sup>14</sup> and the clathrate shells around hydrophobic molecules<sup>8</sup> as a population of predominantly five- and six-membered circular H-bonds.

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## Calcium-dependent regulation of protein synthesis and degradation in muscle

MUSCLE proteins undergo continuous intracellular turnover as do proteins in other cells<sup>1,2</sup>. Furthermore, hormones, nutrients and work load can alter rates of protein synthesis and degradation in muscle, resulting in growth or atrophy of these tissues<sup>1,2</sup>. In hereditary muscular dystrophies, where there is prominent wasting of the affected tissues, rates of both total protein synthesis and degradation are elevated<sup>3–10</sup>. The immediate cause of this muscle atrophy is the imbalance resulting from an increase in protein degradation which exceeds a smaller enhancement in average protein synthesis. No simple explanation based on a defect in the response of dystrophic cells to known hormonal or nutritional factors has satisfactorily explained the elevation in the rates of both protein synthesis and degradation. We have now investigated the possible role of increased cellular  $\text{Ca}^{2+}$  as a mediator of such changes in protein metabolism based on other known structural and biochemical alterations in dystrophic muscles<sup>11,12</sup>. Lesion(s) involving membranes in muscle as well as other cells occur in hereditary dystrophies, including the main human form, Duchenne dystrophy<sup>11,12</sup>. One characteristic of the dystrophic plasma membrane seems to be an increased permeability to the high concentrations of  $\text{Ca}^{2+}$  normally present in extracellular fluid<sup>11,12</sup>. In addition, studies have suggested a decreased ability of sarcoplasmic reticulum to sequester  $\text{Ca}^{2+}$  in dystrophic muscles<sup>13,14</sup>. Thus, it is possible that increased  $\text{Ca}^{2+}$  might be responsible for the stimulation of both protein synthesis and degradation which occurs in these muscles. To test this idea, we have experimentally increased the uptake of external  $\text{Ca}^{2+}$  into rat muscles by using the divalent cation ionophore, A23187.

The ability of this ionophore to increase the transport of  $\text{Ca}^{2+}$  across membranes has resulted in its application as a widely used tool for the study of many  $\text{Ca}^{2+}$ -dependent cellular processes<sup>15</sup>. The experiments reported here demonstrate that increased movement of  $\text{Ca}^{2+}$  into muscle can produce effects which closely resemble dystrophic muscle and that the increased net catabolism can be reversed by certain factors.

We have studied the effect of A23187 in paired, contralateral rat soleus muscles incubated *in vitro* in defined media. Previous studies have shown that such isolated muscle preparations remain viable and maintain linear rates of both protein synthesis and degradation for at least several hours<sup>16,17</sup>. In these conditions, when tension is not maintained, rates of degradation exceed synthesis. For measurements of protein synthesis, incorporation of  $^{14}\text{C}$ -tyrosine into total protein was measured. This amino acid was chosen because it is not metabolised in muscle and does not itself alter the rates of protein synthesis or degradation<sup>16</sup>. To determine absolute rates of synthesis from  $^{14}\text{C}$ -tyrosine incorporated into protein, it was assumed that the intracellular tyrosine-specific activity reflected the tRNA precursor pool. This assumption is valid when high levels of tyrosine (0.5 mM) are added to the medium causing its specific activity to approach that of the intracellular pools<sup>18</sup>. When A23187 ( $10\text{ }\mu\text{g ml}^{-1}$ ) was added to the medium, rates of total protein synthesis were increased by 44% (Table 1). This concentration of A23187 produced maximal stimulation of both protein synthesis and degradation. Addition of A23187 to muscles incubated in calcium-free medium did not affect protein synthesis, indicating a mode of action involving movement of extracellular  $\text{Ca}^{2+}$  into these cells.

Protein degradation was measured in two ways. In one method, net protein degradation was first determined as the amount of total tyrosine released with time into the muscle pools and medium<sup>16</sup>. The absence of metabolism of this amino acid means that the production of free tyrosine must represent the difference between proteolysis and protein synthesis. Addition of A23187 increased tyrosine release by 114% (Table 1). As protein synthesis is elevated in such conditions (Table 1), this increase in released tyrosine actually represents an underestimate of the absolute increase in the amount of protein degradation occurring. To determine rates of protein breakdown, the rates of synthesis and net degradation can be summed. This quantity increased by 71% on addition of the ionophore. An alternative method of measuring degradation involves the addition of cycloheximide to eliminate re-utilisation of amino acids<sup>16</sup>. Although cycloheximide may reduce absolute rates of degradation, the regulatory effects of various physiological factors on proteolysis can still be measured<sup>1,16</sup>. In the presence of cycloheximide, A23187 stimulated tyrosine release by 43% (Table 1).

We then carried out experiments to characterise the increased protein breakdown caused by the  $\text{Ca}^{2+}$  ionophore. As in our experiments on protein synthesis, A23187 had no effect on rates of proteolysis in the absence of extracellular  $\text{Ca}^{2+}$  (Table 1), supporting a mechanism requiring movement of  $\text{Ca}^{2+}$  into the muscle cells. Interestingly, removal of extracellular  $\text{Ca}^{2+}$  by itself reduced degradation. To study the degradation which occurs with elevated intracellular  $\text{Ca}^{2+}$ , we tested the effect of leupeptin on proteolysis (Table 2). This non-toxic, highly specific protease inhibitor has been previously shown to retard muscle degeneration, proteolysis and myofibrillar disassembly without affecting protein synthesis<sup>19–21</sup>. Addition of leupeptin inhibited the elevated rates of degradation which occurred in the presence of A23187 by 32% (– cycloheximide) and 21% (+ cycloheximide). A similar degree of inhibition was also seen in the absence of ionophore, as described previously<sup>20</sup>.

Several studies have reported that protein synthesis and/or degradation in isolated muscles or muscle cells may be influenced by electrical stimulation and even passive stretch<sup>2,22–27</sup>. As such processes also affect the levels of free intracellular  $\text{Ca}^{2+}$ , we investigated the relationship of tension to our ionophore experiments. Muscles were either incubated in a

**Table 1** Effect of A23187 on protein synthesis and degradation in the presence (a) and absence (b) of calcium

Treatment	Protein synthesis (nmol Tyr incorporated per mg per h)		Net protein degradation (nmol Tyr released per mg per h)		Protein degradation (+cycloheximide) (nmol Tyr released per mg per h)	
a						
Control	0.186 ± 0.015		0.121 ± 0.008		0.182 ± 0.015	
+A23187	0.267 ± 0.006	+44%*	0.259 ± 0.015	+114%*	0.260 ± 0.004	+43%†
b						
Control	0.173 ± 0.009		0.084 ± 0.004		—	
+A23187	0.181 ± 0.015	+5% (NS)	0.082 ± 0.003	-2% (NS)	—	

a, Normal female rats (40–55 g) were killed by cervical dislocation and paired soleus muscles immediately removed with tendons intact and weighed. Each muscle was preincubated in the presence or absence of  $10 \mu\text{g ml}^{-1}$  A23187 (Lilly) for 1 h at  $37^\circ\text{C}$ . The incubation medium (3.0 ml) also contained Krebs–Ringer bicarbonate solution saturated with 95%  $\text{O}_2$ –5%  $\text{CO}_2$  and supplemented with 10 mM glucose, 5× plasma levels of the branched chain amino acids (valine, isoleucine and leucine), and 0.1 unit  $\text{ml}^{-1}$  insulin<sup>16</sup>. A23187 was dissolved in ethanol and the final concentration of ethanol in all incubations was adjusted to 0.5% v/v. This amount of ethanol did not affect protein synthesis or degradation. After 1 h, the muscles were blotted and transferred to fresh medium of the same composition but containing in addition  $^{14}\text{C}$ -tyrosine (0.06  $\mu\text{Ci ml}^{-1}$ ) and unlabelled tyrosine (0.5 mM). The muscles were incubated for 1 h at  $37^\circ\text{C}$  and then placed in 10% trichloroacetic acid and protein synthesis was measured as described previously<sup>16</sup>. Determination of synthesis was carried out by correcting rates of  $^{14}\text{C}$ -tyrosine incorporation into acid-precipitable protein using the specific activity of the intracellular tyrosine pool<sup>16</sup>. For measurement of net protein degradation muscles were incubated without tyrosine to allow accurate measurement of the release of free tyrosine into the muscle pools and medium. Omission of tyrosine has no effect on rates of muscle protein synthesis or degradation<sup>16</sup>. After 1 h of preincubation the tyrosine content of the muscle pools was in a steady state in the presence or absence of ionophore; therefore, net protein degradation measured during the second hour was proportional to tyrosine appearing in the medium during that period. Tyrosine was measured fluorometrically as previously described<sup>16</sup>. Protein degradation was measured similarly except that cycloheximide (0.5 mM) was added to both the preincubation and incubation media. b, Muscles were treated as in a except that the Krebs–Ringer bicarbonate buffer was prepared without calcium but containing the calcium chelator EGTA (2 mM). Results are expressed as the mean value  $\pm$  s.e.m.,  $n = 6$ .  $P$  values were calculated for the paired muscle differences using the Student  $t$ -test. NS, not significant.

\*  $P < 0.005$ .†  $P < 0.003$ .

flaccid state as in Tables 1 and 2 or were incubated with slight passive tension by pinning the tendons so the muscle was maintained at approximately 110% of rest length. When muscles were incubated under tension without ionophore, no effect on synthesis was detected (Table 3). Such results confirm earlier studies with the adult soleus which showed that active or passive tension did not increase synthesis in similar conditions<sup>2,25</sup>. In contrast, fixing the length of the soleus in the presence of A23187 caused a further (23%) increase in protein synthesis above that caused by the ionophore alone (Table 3). Although passive tension alone had no effect on synthesis, as expected, the addition of A23187 to pinned muscles produced a large increase in synthesis (70%) equal to the sum of the above two effects (Table 3).

The increase in synthesis produced with A23187 may result from higher average intracellular  $\text{Ca}^{2+}$  levels than those which occur on intermittent stimulation or stretch as described previously<sup>2,25</sup>. Stimulation may, however, produce higher transient  $\text{Ca}^{2+}$  increases, which would explain the greater peak tension produced with stimulation than A23187 in the soleus<sup>28</sup>. Possibly, more frequent stimulation and/or stretch applied for longer periods further increases free  $\text{Ca}^{2+}$  and may account for several reports of increased protein synthesis caused by stimulation or stretch<sup>22–24,26</sup>. In any case, the ability of A23187 to stimulate protein synthesis is seen when the muscles are flaccid, contracting against no load, indicating that the  $\text{Ca}^{2+}$ -dependent increase in synthesis does not require the development of tension. The further enhancement of synthesis seen when ionophore was added to muscles incubated with slight stretch may result from higher  $\text{Ca}^{2+}$  levels produced by longer sarcomere lengths<sup>29</sup> or possibly length-dependent effects on the sarcolemma. Related studies have shown that most of the  $\text{Ca}^{2+}$ -dependent protein synthesis requires RNA synthesis (G. Silver and J.D.E., in preparation).

In contrast to the potentiating effect of tension on  $\text{Ca}^{2+}$ -dependent synthesis, the effect of tension on degradation was opposite to that produced by A23187. Table 3 shows that maintaining muscle tension decreased rates of proteolysis in the presence of A23187. Furthermore, unlike the effect of tension on synthesis (which was only seen in the presence of ionophore), maintaining the muscle under slight stretch also produced a similar inhibition of degradation in the absence of A23187, as previously reported by Goldberg *et al.*<sup>2,25</sup>. Although the mechanisms by which muscle stretch or activity reduce proteolysis are unclear, the present results argue against a  $\text{Ca}^{2+}$ -mediated effect. Further support for this conclusion comes from the observation that, even over a wide range of ionophore concentration, inhibition of degradation was not detected in the absence of tension (data not shown).

There are several possible mechanisms which can explain the

$\text{Ca}^{2+}$ -dependent stimulation of proteolysis.  $\text{Ca}^{2+}$  may modify the structure of muscle proteins, rendering them more susceptible to proteolysis, or it may stimulate the activity of cellular proteases. Activation of proteolysis could involve an increase in the number of muscle lysosomes, leakage of lysosomal enzymes, or increased uptake of proteins into this organelle. Alternatively, mammalian cells are known to contain soluble neutral proteases<sup>30–33</sup>, and  $\text{Ca}^{2+}$ -dependent protease(s) from muscle seem to be capable of initiating myofibrillar degradation and hydrolysing other proteins<sup>31–33</sup>. Direct activation of such non-lysosomal proteases seems an attractive explanation of the ionophore effect on degradation rates, although other indirect mechanisms cannot be excluded.

The actual mechanisms involved in the  $\text{Ca}^{2+}$ -dependent effects described here are unclear, but several factors indicate that they do not involve gross toxic effects on cells. First, elevation of protein synthesis in the presence of the ionophore indicates good viability, as this process is sensitive to small drops in ATP levels. Also, exposure of the muscles to A23187 for 2 h produced no loss of subcellular structure or ability to produce maximum twitch tension on electrical stimulation (T. K., D. Erlij and J.D.E., in preparation). Finally, in previous studies with this muscle no alteration in resting potential was seen with the same concentrations of A23187 ( $10 \mu\text{g ml}^{-1}$ ) producing maximal effects in the present studies<sup>28</sup>. These results differ considerably from the results of certain workers using this ionophore in other systems. For example, studies with frog skeletal muscle have reported contraction accompanied by a decrease in resting potential and dramatic destruction of subcellular structure on exposure to A23187<sup>34</sup>. Such effects may be related to our observations of decreased protein synthesis seen in certain muscle preparations or upon longer exposure to ionophore (data not shown).

**Table 2** Effect of leupeptin on protein degradation in the absence (a) and presence (b) of A23187

Treatment	Net protein degradation (nmol Tyr released per mg per h)		Protein degradation (+cycloheximide) (nmol Tyr released per mg per h)	
a				
Control	0.139 ± 0.018		0.208 ± 0.011	
Leupeptin	0.073 ± 0.007	-47%*	0.162 ± 0.006	-22%*
b				
Control	0.181 ± 0.007		0.217 ± 0.009	
Leupeptin	0.123 ± 0.013	-32%†	0.171 ± 0.007	-21%‡

Muscles were incubated as described in Table 1. Cycloheximide (0.5 mM) and leupeptin (50  $\mu\text{M}$ ) were added to both the preincubation and incubation medium as indicated.

\*  $P < 0.01$ .†  $P < 0.001$ .‡  $P < 0.005$ .

**Table 3** Effect of tension on protein synthesis and degradation

Treatment	Protein synthesis (nmol Tyr incorporated per mg per h)	Protein degradation (+cycloheximide) (nmol Tyr released per mg per h)
<i>a</i>		
Control	0.299 ± 0.034	0.275 ± 0.012
Stretch	0.286 ± 0.016	0.200 ± 0.011
<i>b</i>		
A23187	0.240 ± 0.007	0.417 ± 0.022
Stretch + A23187	0.295 ± 0.017	0.258 ± 0.021
<i>c</i>		
Stretch	0.243 ± 0.024	—
Stretch + A23187	0.412 ± 0.022	—

Muscles were maintained under tension throughout the preincubation and incubation periods by fixing the length of the tissues in a stretched position (110% of rest length) with pinning through the tendons into small plastic supports. Other muscles were incubated in a flaccid state. Ionophore ( $10 \mu\text{g ml}^{-1}$ ) was added as indicated and measurements were carried out as described in Table 1. Differences between mean values for identical conditions tested during different experiments (*a*, *b*, *c*) illustrates day to day variations and the advantage of paired muscle analysis.

\*  $P < 0.025$ .

†  $P < 0.01$ .

‡  $P < 0.003$ .

In related studies we have found that combinations of different degrees of stretch and  $\text{Ca}^{2+}$  can produce effects consistent with muscle hypertrophy as well as atrophy (J.D.E., T.K. and K. Matsumoto, in preparation). However, the observations reported here closely resemble the hereditary muscular dystrophies. Thus,  $\text{Ca}^{2+}$  may be directly responsible for at least a substantial part of the increased protein turnover and net negative nitrogen balance characteristic of muscular dystrophies. Tension seems to be particularly effective in decreasing  $\text{Ca}^{2+}$ -induced net catabolism by increasing synthesis as well as decreasing degradation, and leupeptin seems able to decrease proteolysis in the ionophore-treated muscles. Thus, maintenance of passive tension, and other agents like leupeptin and possibly calcium chelators may be of therapeutic value in the treatment of certain muscular dystrophies.

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## Cloning and endonuclease mapping of the hepatitis B viral genome

THE virus that causes hepatitis B, or serum hepatitis, seems to infect only humans in nature, and experimental infection has been achieved in only a few additional mammals. The limited host range of the hepatitis B virus (HBV), and its failure so far to infect tissue culture cells have drastically restricted study of this virus and have hindered development of a vaccine for the serious disease that it causes. We report here the cloning of double-stranded HBV DNA in *Escherichia coli* K12, using the unique *EcoRI* cleavage site on the viral genome to introduce the entire HBV DNA molecule into an *EcoRI* cleavage site within the chloramphenicol (Cm) resistance gene of the pACYC184 plasmid vector<sup>1</sup>.

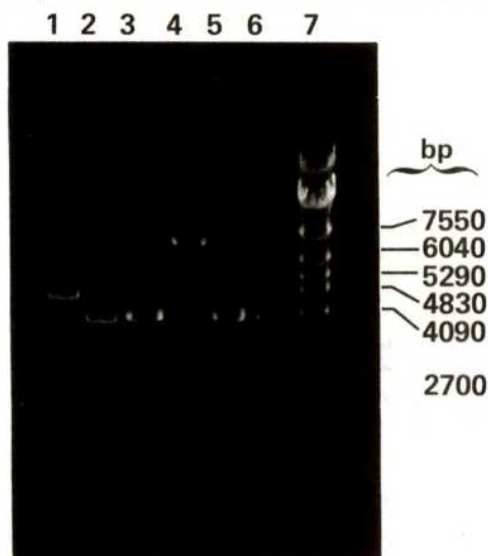
The Dane particle<sup>2</sup>, a particulate form of viral antigen found in the blood of infected patients, seems to be the infectious form of HBV. DNA isolated from Dane particles has a molecular weight of  $2.1 \times 10^6$  (corresponding to an approximate genome length of 3,200 base pairs), and is single-stranded for about 15–45% of its length<sup>3,4</sup>. Using either the endogenous DNA polymerase of the Dane particle<sup>4</sup> or avian myeloblastosis virus RNA-dependent DNA polymerase (reverse transcriptase)<sup>5</sup>, the hepatitis B viral genome can be converted to a double-stranded circular form, which is cleaved by the *EcoRI* endonuclease into a unit length linear structure (A. Siddiqui, F. R. Sattler and W. S. Robinson, in preparation). We have introduced this *EcoRI*-cleaved viral DNA into the *EcoRI* site of the Cm-resistance gene of plasmid pACYC184. The previously reported<sup>4</sup> heterogeneity of Dane particle DNA has been investigated by analysis of multiple separately-cloned DNA molecules, and a restriction endonuclease cleavage map of the HBV genome has been constructed.

The surface antigen of the hepatitis B virus (HBsAg) is known to be antigenically complex; four major subtypes (adw, ayw, adr, ayr) have been identified, and recent immunological evidence suggests that antigenic heterogeneity exists also within each of these subtypes<sup>6</sup>. Siddiqui *et al.* (in preparation) have observed that Dane particle DNA isolated from carriers infected with different hepatitis B viral subtypes show divergent *HincII* and *HaeIII* endonuclease cleavage patterns, and some degree of heterogeneity has been found even within the same subtype. In order to circumvent heterogeneity between different HBsAg subtypes or between different carriers of the same subtype, the DNA used in our initial cloning experiments was isolated from Dane particles of a single HBsAg carrier (subtype adw). This method also allowed us to investigate further the question of possible heterogeneity of hepatitis B viral DNA isolated from a single carrier.

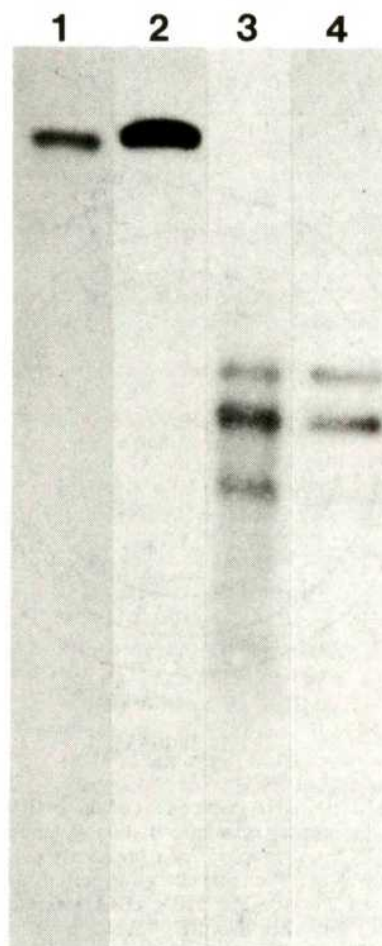
In order to decrease the frequency of recircularisation of the pACYC184 vector plasmid and to maximise formation of composite plasmids, *EcoRI*-cleaved pACYC184 DNA was treated with alkaline phosphatase<sup>7</sup> before addition of *EcoRI*-



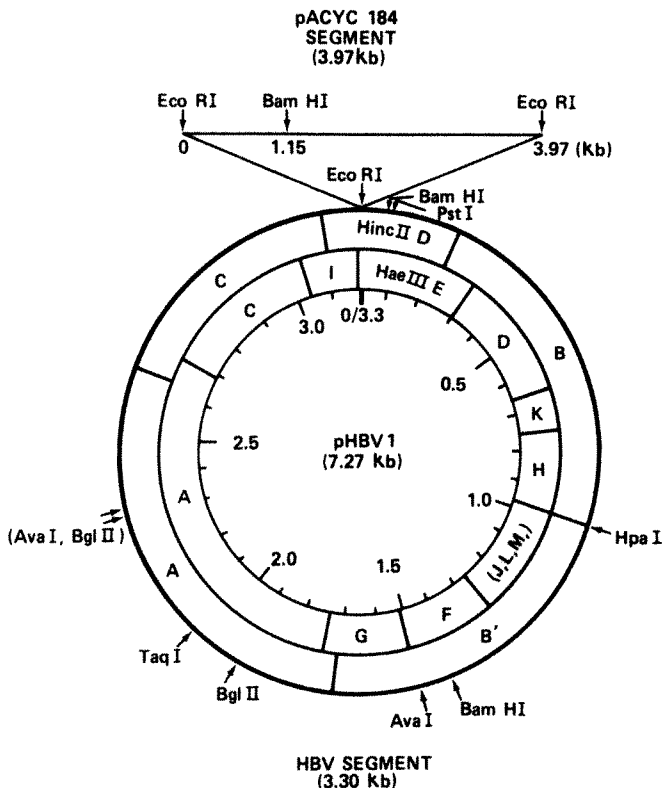
treated Dane particle DNA that had been made double-stranded using the endogenous DNA polymerase. The DNA species were ligated and introduced by transformation into a non-restricting ( $r_{km}^+$ ) mutant *E. coli* C600 (ref. 8); bacteria were plated on nutrient agar plates containing tetracycline (Tc,  $10 \mu\text{g ml}^{-1}$ ) 40 min after the DNA uptake step of the transformation procedure to ensure that individual transformants



**Fig. 1** Cloning and restriction endonuclease analysis of recombinants between Dane particle DNA and the pACYC184 plasmid vector. Dane particle DNA was isolated from the plasma of a chronic hepatitis B surface antigen (subtype adw) carrier 1083 and made fully double-stranded using the endogenous polymerase<sup>4</sup>. 100 ng of this DNA was digested with 0.5 units of *Eco*RI endonuclease (New England Biolabs) for 30 min as previously described<sup>8</sup>. pACYC184 DNA 2  $\mu\text{g}$  isolated as described elsewhere<sup>12</sup> was simultaneously digested with a twofold excess of endonuclease *Eco*RI and treated with 0.2 U of calf intestine alkaline phosphatase (Boehringer) to prevent recircularisation during the subsequent ligation<sup>7</sup>. The reaction was stopped by heating at  $65^\circ\text{C}$  for 10 min, followed by phenol extraction. After precipitation by ethanol, the phosphatase-treated plasmid DNA was mixed with the *Eco*RI-cleaved fully double-stranded Dane particle DNA in a final volume of 50  $\mu\text{l}$ , and ligated using 0.14 U of bacteriophage T4 DNA ligase (New England Biolabs) for 8 h at  $12.5^\circ\text{C}$  in standard conditions<sup>8</sup>. The reaction mixture was added directly to the  $\text{CaCl}_2$ -treated *E. coli* C600 ( $r_{km}^+$ ) for transformation<sup>8</sup>. P3+EK1 containment conditions were used, as specified in the 22 December 1978 recombinant DNA guidelines of the US National Institutes of Health. Transformants were selected on Penassay base agar (antibiotic medium 2, Difco) containing Tc ( $10 \mu\text{g ml}^{-1}$ ). After 24 h at  $37^\circ\text{C}$ , colonies were screened for insertions in the gene that codes for chloramphenicol resistance<sup>1</sup> by streaking clones on plates containing Cm ( $25 \mu\text{g ml}^{-1}$ ). Plasmid DNA was isolated from those clones which were sensitive to Cm as described<sup>12</sup>. Endonuclease digests of one representative plasmid designated pHBV1 were analysed by gel electrophoresis. Vertical agarose (0.7%, Seakem, M.C.I.) gels (20 cm long) were run at 35 mA (ref. 9). pACYC184 and pHBV1 DNAs were treated with the indicated endonucleases and molecular lengths were calculated using *Eco*RI-generated fragments of pRR12 as a size standard (see below) and are accurate within  $\pm 10\%$  (ref. 13). Lanes: (1) pACYC184 DNA, showing uncleaved superhelical dimers and higher multimers; (2) pHBV1 recombinant DNA, showing uncleaved superhelical monomers and higher multimers; (3) *Hind*III-cleaved pACYC184 DNA, showing a single linear fragment (3.97 kilobases); (4) *Hind*III-cleaved pHBV1 recombinant DNA, showing linear fragment (7.91 kilobases), as HBV DNA is not cleaved by this enzyme; (5) *Eco*RI-cleaved pACYC184 DNA, showing single linear fragment (3.97 kilobases); (6) *Eco*RI-cleaved pHBV1 showing two fragments (3.97 and 3.3 kilobases); (7) *Eco*RI-generated fragments of plasmid pRR12 used as molecular length standards<sup>13</sup>. The lengths of the fragments shown are (from top to bottom) 20.39, 12.08, 11.40, 10.88, 7.55, 6.04, 5.29, 4.83, 4.09, 2.70, 1.66, 1.53 and 1.13 kilobases.



**Fig. 2** Autoradiogram of pHBV1 DNA after endonuclease digestion and hybridisation with a nick-translated Dane particle DNA probe. The fractionation of endonuclease cleaved DNA was as described in Fig. 1, except that a 1.5% agarose gel was used. The DNA was transferred from the agarose slab gels onto nitrocellulose filters (Millipore) by the method of Southern<sup>14</sup>. After the transfer, the filter was incubated for 16 h in a  $6\times\text{SSC}$  solution containing prehybridisation buffer (0.02% each of Ficoll, polyvinylpyrrolidone and bovine serum albumin)<sup>15</sup>. Dane particle DNA was labelled by nick translation<sup>16,17</sup>. The specific activity of the DNA probe was  $5.09\times 10^7$  c.p.m.  $\mu\text{g}^{-1}$ . Initially, the  $^{32}\text{P}$ -labelled DNA ( $1.5\times 10^6$  c.p.m.) was denatured in a boiling water bath for 20 min in the presence of  $1.3 \text{ mg ml}^{-1}$  sonicated salmon sperm DNA (total volume 1.2 ml), chilled briefly, then brought to a final volume of 5 ml hybridisation buffer containing 50% formamide,  $5\times\text{SSC}$ , and 0.2% sodium lauryl sulphate. After removing the prehybridisation buffer, the filter ( $8\times 15 \text{ cm}$ ) was annealed with the HBV probe for 12 h at  $42^\circ\text{C}$  in a sealed plastic bag. It was then washed twice for 1 h each time at  $42^\circ\text{C}$  with 25 ml of the hybridisation buffer and repeated an additional two times with 100 ml  $2\times\text{SSC}$ , air dried and exposed to an X-ray film in the presence of a Dupont Cronex intensifying screen at  $-70^\circ\text{C}$  for 2 h. Lanes: (1) *Eco*RI-cleaved  $^{32}\text{P}$ -labelled Dane particle DNA made double-stranded with endogenous polymerase, to serve as a marker for the location of unit length HBV DNA; (2) *Eco*RI-cleaved pHBV1 DNA; (3)  $^{32}\text{P}$ -labelled nick-translated HBV DNA that has been cleaved with both *Eco*RI and *Hinc*II, to serve as a marker for the sizes of the endonuclease generated fragments from the genome; (4) pHBV1 DNA cleaved with both *Eco*RI and *Hinc*II. Lanes 1 and 3 were exposed to X-ray film before hybridisation was performed. The 94-base pair fragment resulting from the *Eco*RI cleavage of the *Hinc*II 'D' fragment (see Fig. 3) does not appear in the autoradiogram because it is not retained by this gel. The 220-base pair fragment resulting from this same cleavage of *Hinc*II fragment D does not appear in lane 4 because it is incompletely retained on the filters during hybridisation in the conditions used.



**Fig. 3** Map of HBV DNA component of the pHBV1 plasmid, showing cleavage sites for certain restriction endonucleases. The cloned HBV DNA was mapped by a previously described procedure which involves  $^{32}\text{P}$ -end labelling and partial enzyme digestions<sup>10</sup> (data not shown). The HBV DNA insert contains no cleavage sites for the *Hind*III, *Sal*I, *Sma*I, or *Xho*I endonucleases. The *Eco*RI cleavage site was arbitrarily chosen as the zero position for the map.

would represent separate clones. Tc-resistant transformants were selected and screened for resistance to Cm. Over 90% of those clones resistant to Tc were sensitive to Cm, indicating interruption of the continuity of the Cm resistance gene; plasmids from 25 of these were isolated and examined by agarose gel electrophoresis<sup>9</sup> after *Eco*RI cleavage. Twenty of the cloned plasmids were found to contain a single DNA insert approximately 3,300 base pairs in length (Fig. 1); the remaining five plasmids had undergone deletion of vector DNA in the vicinity of the *Eco*RI cleavage site. Using *Bam*HI endonuclease, which cleaves the pACYC184 and HBV DNA molecules asymmetrically, we found that both orientations of HBV DNA insertion were equally represented in these clones (data not shown).

The identity of the DNA insertions within pACYC184 was verified by cleavage of a selected composite plasmid (pHBV1) by several different site-specific endonucleases and by hybridisation of radioactively-labelled HBV DNA to the endonuclease-cleaved recombinant plasmid (Fig. 2). In addition, to facilitate further studies of the structure of the HBV genome and the proteins that it encodes, an endonuclease cleavage map (Fig. 3) of the pHBV1 plasmid was constructed using the procedure of Smith and Birnstein<sup>10</sup>. The location of endonuclease cleavage sites within cloned pHBV1 DNA is in agreement with a preliminary map (Siddiqui *et al.*, in preparation), constructed for HBV DNA isolated directly from Dane particles obtained from the same human carrier. This finding provides further verification of the nature of the DNA insert contained in the pHBV1 plasmid, and indicates also that the entire HBV genome is present and stably maintained in the recombinant plasmid. Experiments reported recently by Fritsch *et al.*<sup>11</sup> indicate that recombinants between HBV DNA and bacteriophage  $\lambda$ gtWES.4B can also be propagated stably in *E. coli* K12.

Landers *et al.*<sup>4</sup> reported that the sum of fragments produced by *Hae*III endonuclease cleavage of Dane particle HBV DNA is greater than the molecular size of the entire virus, and concluded that the DNA molecules contained in different Dane molecules were not identical. In our studies of HBV DNA cloned in *E. coli* K12, we have observed that the sum of the sizes of the *Hae*III fragments in 20 separate clones equals exactly the molecular weight of the virus. The difference between the fragmentation patterns observed by us for *Hae*III-digested cloned HBV DNA, and the pattern reported by Landers *et al.*<sup>4</sup> using Dane particle DNA obtained from a different carrier resides partly in the two largest *Hae*III-generated fragments of the virus; *Hae*III cleavage of the DNA studied by Landers *et al.* yielded a doublet with components estimated to be 986 and 957 base pairs. Electrophoresis of the cloned DNA showed a single fragment having a mobility consistent with a size of 986 base pairs, as did fully-stranded Dane particle DNA isolated from that carrier. Electrophoresis of a mixture of *Hae*III-cleaved pHBV1 DNA with identically digested DNAs from 19 other HBV-pACYC184 recombinant plasmids we have studied showed no double band at the relevant location in the gel.

In addition, the cloned DNA does not show the 750-base pair *Hae*III-generated fragment found in Dane particle DNA by Landers *et al.*<sup>4</sup> Since this fragment appeared only in Dane particle DNA that was elongated with endogenous DNA polymerase in the presence of deoxyribonucleoside triphosphates, its presence may have resulted from incomplete 'fill in' of the single-stranded portion of the HBV genome.

The ability to clone the total genome of HBV in an *E. coli* K12 strain now allows the amplification and propagation of hepatitis viral DNA species isolated from individual carriers and the production of substantial quantities of HBV DNA for further structural studies. Investigation of endonuclease cleavage patterns of cloned Dane particle DNA derived from different carriers showing the same viral subtype, and from individuals infected with different subtypes, should elucidate the extent of heterogeneity of the HBV genome in such carriers and should enable correlation of DNA primary structure with subtype specificity.

In addition, the prospect of expression in bacterial cells of peptides encoded by the cloned HBV viral genome potentially provides a means of synthesising large amounts of viral surface antigen suitable for use in production of a vaccine for viral hepatitis.

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# matters arising

## Improved amorphous semiconductors for solar cells

OVSHINSKY AND MADAN<sup>1</sup> have described the interesting results of incorporating fluorine in glow discharge deposited amorphous silicon (in particular a very low density of states within the gap.) However, as one would expect on general grounds, fluorinated material has a significantly larger energy gap than hydrogenated material. It therefore offers an even less good match to the solar spectrum, which would not improve the efficiency of solar cells made from it. Although the use of iodine rather than fluorine might be expected to be helpful in this respect it is still unlikely to reduce the energy gap to the near 1 eV value required for optimum spectral response. [The stability of the Si-I bond shown by the surface stabilisation of silicon by iodine<sup>2</sup>, led me to discuss the possible use of iodine in glow discharge deposited silicon with several workers at the International Conference on the Physics of Semiconductors at Edinburgh in September 1979.]

During our group's early work on glow discharge deposition of amorphous materials (see refs 3, 4) it was observed that germanium and silicon-germanium alloys deposited by glow discharge from germane or germane-silane mixtures, even with rather small germanium content, show negligible photoconductivity compared with pure silicon. This would now be interpreted in terms of the Ge-H bond being weak compared with the Si-H bond, with the result that dangling bonds on germanium atoms are not stably hydrogenated in amorphous germanium and its alloys, and with the further consequence that these materials have a large density of states in the gap. It would therefore not be possible to prepare solar cells from them even though their absorption edge could be well matched to the solar spectrum. Clearly this situation would alter if a stronger bonding element, such as a halogen, were used to satisfy germanium dangling bonds. (Even the Ge-I bond is far more stable than Ge-H). What is suggested, then, is that particularly promising amorphous semiconductors for making efficient solar cells would be germanium and Ge-Si alloys incorporating halogen, the overall composition tailored to optimise the photoconductive response spectrum to solar radiation.

*Note added in proof:* The optimum energy gap for matching a cell to the AM1 solar spectrum is about 1.4 eV (see, for example, ref. 5). However, even at the Equator the Sun is not long at the zenith (implied by AM1), and the increasing atmospheric absorption at lower solar angles characteristic of most of the day (all of it at higher latitudes, outside the tropics) shifts the optimum energy gap to still lower values. It would only be with high temperature operation (above ~300 °C) that fluorinated amorphous silicon could be considered to provide a good spectral match. As the main aim in developing large area solar cells is to avoid concentration of radiation, the maximum temperature likely to be encountered with them is perhaps 65 °C.

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OVSHINSKY AND MADAN REPLY—Goodman suggests that (1) the optimal energy gap for solar photovoltaic energy conversion is 1 eV and (2) fluorinated amorphous silicon has a significantly larger energy gap than hydrogenated amorphous silicon, and thus concludes that amorphous germanium or Ge-Si alloys are preferential to amorphous silicon for solar-cell applications. Both of the above premises are incorrect.

It is well known<sup>1,2</sup> that the optimal gap for solar photovoltaic energy conversion is about 1.6 eV, not 1 eV. For example, the maximum efficiency for Am-0 using a semiconductor with an energy gap of 1.6 eV is about 25%, more than half again as large as the 16% maximum efficiency of a material with a 1.0 eV gap<sup>3</sup>. Furthermore, the energy gap of our new amorphous Si:F:H alloy is determined by a Fowler plot and clearly stated in our letter<sup>4</sup> is 1.65 eV, which is essentially the optimal value. In fact, it is not significantly different from the energy gaps of 1.55–1.8 eV obtained in silane-decomposed films<sup>5,6</sup>, nor is it much larger than the 1.5 eV gap of nominally pure amorphous silicon<sup>7,8</sup>. The reason for this is that the

fluorine concentration of the new alloy is <5% and thus the vast majority of the chemical bonds that determine the energy gap of the material are ordinary Si-Si covalent bonds.

The major purpose of our letter<sup>4</sup> was to point out that the new alloy has many features in addition to its band gap that makes it more desirable than silane-decomposed material for solar-cell applications.

*Note added in proof:* Goodman in his note in proof now suggests that higher air-mass conditions would greatly deteriorate the performance of amorphous Si:F:H solar cells on the basis of a 1.65-eV gap. The facts are as follows<sup>9</sup>. At AM-2, when the sunlight is at an angle of 60° to the Earth's surface (approximately the spectrum for average, slightly hazy weather conditions and smaller sun angles, as in temperate-zone climates), the maximum conversion efficiency of an ordinary solar cell is ~26%. For a gap of 1.65 eV, the maximum conversion efficiency is still 25%. This figure, in fact, is larger than the maximum conversion efficiency at any energy gap for AM-0 conditions (because of the decrease in relative solar irradiance in the near infrared at AM-2). The main point is that the maximum conversion efficiency is only a weak function of energy gap in the 1.0–2.0 eV range (never dropping below 20% in this range at AM-2), so that the efficiencies of real devices are much more dependent on other parameters.

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## Asbestos-enhanced uptake of carcinogens

THE results of Lakowicz and Hylden's studies<sup>1</sup> on asbestos-mediated uptake of benzo(a)pyrene (BP) by dipalmitoyl L- $\alpha$ -phosphatidylcholine (DPPC) vesicles are more indicative of *in vivo* transfer of BP from particulates to lung surfactant (mainly DPPC and other phospholipids<sup>2</sup>)

than transfer of BP from particulates to biological membranes (mainly phospholipids and globular proteins<sup>3</sup>). As a monomolecular layer of surfactant containing DPPC exists at the air-liquid interface in the lung, BP adsorbed onto inhaled particulates would be at least partially transferred to lung surfactant before transfer to cell membranes would be initiated.

I suggest that the reported<sup>1</sup> increased rate of transfer for BP adsorbed to particulate silica or amosite asbestos as compared with BP in a microcrystalline form is principally a result of the adsorption of the mobile DPPC vesicles onto the particulate surface, rather than increased solubilisation of BP in an aqueous phase. It has been shown that DPPC readily adsorbs onto the surface of glass fibres and various types of asbestos, including amosite<sup>4</sup>. The adsorption is controlled by coulombic interactions between the ionic head group of the DPPC and the electrostatic charge of the particulates. The particulate surface charge also is significant in determining particulate-membrane interactions<sup>4</sup>. While the particulate surface charge controls DPPC adsorption, the particulate specific surface area and the form of the adsorbed BP (such as microcrystalline or monomeric<sup>1</sup>) determine the surface area covered with BP at the particulate-liquid interface and thus the availability of BP for transfer to adsorbed DPPC or cell membranes. The interfacial availability of BP would govern relative transfer rates for particulates, such as silica and amosite, which display essentially the same surface charge<sup>4</sup>. On the basis of these comments, I suggest that the preferential interfacial transfer of carcinogens to cell membranes and/or lung surfactant at the particulate surface may be an important factor in co-carcinogenesis.

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LAKOWICZ ET AL. REPLY—we are aware that DPPC is a major component of lung surfactant; however, it is not known whether carcinogens which are adsorbed to particles are eluted during contact with surfactant or after subsequent contact with cells. For this reason we are investigating the effects of asbestos and silica on uptake of BP into natural membranes, namely rat liver microsomes and 3T3 cells. Again we find that these particles enhance BP uptake and that asbestos is

more effective than silica. Thus, it is clear that the particle-enhanced uptake of BP is a more general phenomenon which could occur not only in lung surfactant but also in lung cells.

The mechanism of the transfer process has not been elucidated. We suggested that the particle enhancement of uptake results from an increased rate of solubilisation of the BP in the aqueous medium, whereas Light proposes that the transfer of the BP is to vesicles which have become bound to the particulates. The basis for our argument comes from more detailed studies of the particle-enhanced uptake of another polynuclear aromatic hydrocarbon (PAH), 1,2-benzanthracene, into DPPC vesicles<sup>1</sup>. In that instance we measured virtually the same uptake kinetics over a wide range of particle to DPPC ratios. For this reason we propose that the enhanced uptake results from an increased rate of solubilisation of these PAH by virtue of their association with the particulate materials. We do not intend to indicate that the particles can increase the equilibrium solubility of the PAH.

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## The evidence for species guilds is an artefact

THE evidence cited by McNaughton<sup>1</sup> that ecosystems are loosely linked sets of species guilds seems to be based on an unfortunate artefact. Although the independent occurrence of species in samples is appropriately tested using the point correlation coefficient ( $V$ ), it is effectively precluded by the sampling method used. This leads to a non-zero expectation for the point correlation coefficient for species whose spatial distributions are independent.

The dependence introduced by the sampling method may be demonstrated analytically. If an area occupied by a number of species distributed independently in space is sampled and the resulting distributions of the  $a$ th and  $b$ th species ( $A$  and  $B$ ) among samples are independent, then

$$P\{A \cap B\} = P\{A\}P\{B\} \quad (1)$$

Where the events  $A$  and  $B$  are the occurrence in a sample of  $A$  and  $B$ , respectively. However, if it is sampled in the manner described by McNaughton it can be shown that

$$P\{A \cap B\} = P\{A\}P\{B\} - (1 + (k-1) \times (h+1) + k^2 g_a g_b) f_a f_b / (g_a g_b) \quad (2)$$

In this,  $f_i$  is the proportionate cover of the  $i$ th species,  $g_i = 1 - f_i$ , and, summing over all species except  $A$  and  $B$ ,  $h = \sum_i f_i$  and  $k = \sum_i f_i / g_i$ . As  $g_i \leq 1$  for all  $i$ ,  $k \geq h$ , so that equation (2) implies

$$P\{A \cap B\} \leq P\{A\}P\{B\} - h^2(1 + g_a g_b) f_a f_b / (g_a g_b)$$

Hence, as long as  $A$  and  $B$  occur, together with at least one other species (that is  $0 < P\{A \cap B\} < 1$ ),

$$P\{A \cap B\} < P\{A\}P\{B\}$$

This contradicts equation (1). It follows that if two species independently distributed in space are sampled by this method and a point correlation can be calculated between them, its expected value is negative.

Due to its dependence on  $k$ , this bias declines with increased numbers of species as long as the form of the species abundance curve remains consistent. To determine the extent to which this might account for the effects observed by McNaughton, the sampling method was simulated on a computer, the first individual of each sample being selected at random from the species complement, the second at random from all species except that of the first. The average interaction term  $i$  and connectance  $c$  were estimated for sets of 100 sample points in the manner used by McNaughton, calculating  $V$  only when both species were represented by at least 10 individuals.

The extent of the bias in  $V$  depends on the frequency distribution of species abundance. It was postulated that the ranked population frequencies of the

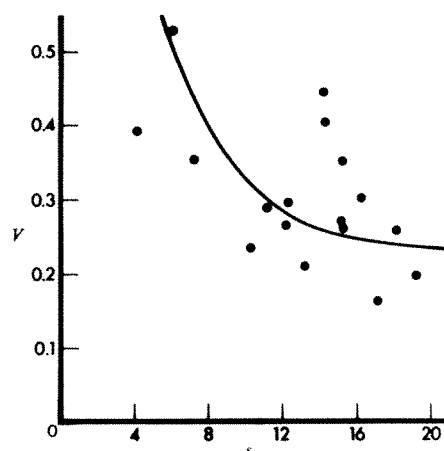


Fig. 1 The relationship between average interaction strength and number of species generated by the use of the sampling method of McNaughton<sup>1</sup> on species distributed independently in communities with log-series species abundance curves (solid line) for comparison with that reported for African grasslands.



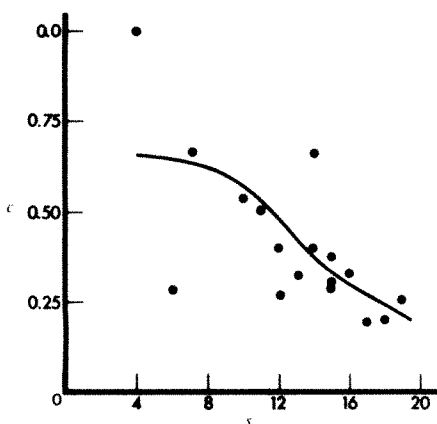


Fig. 2 The relationship between connectance and species number generated with the sampling method of McNaughton from communities with log-series species abundance curves for species distributed independently in space (solid line) for comparison with that reported for African grasslands.

species sampled formed a geometric series, with common ratio  $\exp(-1/\alpha)$ . Random sampling from such a distribution yields a log-series species abundance curve with diversity index<sup>2</sup>  $\alpha$ . Such curves have frequently been found to provide an adequate description of species abundance data, albeit largely for insects<sup>2</sup>, and have the advantage that they are effectively completely determined by sample size and species number.

Mean values of species number ( $s$ ),  $\hat{c}$  and  $\hat{\alpha}$  were determined for various values of  $\alpha$ . The relations of  $\hat{c}$  and  $\hat{\alpha}$  to  $s$  thus obtained are superimposed on the data of McNaughton in Figs 1 and 2. The curves seem to fit the data points adequately, leaving no residual systematic effect, affirming that the apparent decline in these parameters with increased species number is an artefact of the sampling method used.

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## Stability and diversity in grassland communities

In his analysis of the stability properties of large, randomly constructed model ecosystems, May<sup>1</sup> contradicted the existing ecological dogma—that increased community complexity produces increased stability—by showing that in Lotka–Volterra models, dynamic stability is diminished with increases in: (1) the number of species ( $S$ ); (2) the average strength of the interactions among species ( $i$ ); (3) the connectance of the system ( $c$ ), that is, the proportion of non-zero values for  $i$  in the community interaction matrix.

He showed that the system is almost certainly stable if

$$i(Sc)^{1/2} < 1 \quad (1)$$

McNaughton<sup>2</sup> uses data from a study of the interactions among plant species in 17 grassland stands in the Serengeti National Park to test May's results, and concludes that both average strength of interaction and connectance are negatively correlated with species richness, implying that the structure of these grassland communities is constrained in special ways to meet the requirements for local stability. If this were true, it would be an important conclusion<sup>3</sup>. Unfortunately, McNaughton's results are an inevitable consequence of varying  $S$ , and hence say nothing about whether interactions in these communities are constrained by dynamic interactions between species.

McNaughton measures the strength of interaction among the plant species by applying to nearest-neighbour data<sup>4</sup> the point correlation coefficient,  $V$  (giving limits of  $+1$  if the two species always occur

we hope that further analysis of data of this kind will now be attempted by others.

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MCNAUGHTON REPLIES—Lawton and Rallison's statement that "as species richness increases,  $V$  is bound to decrease" is incorrectly applied to my Letter. As I reported negative values for  $V$ , the number of contacts would have increased, not decreased, had my initial report been correct. But it was wrong and Harris accurately identified the flaw.

I am grateful to them for calling attention to my error and hope that other ecologists using  $2 \times 2$  contingency tests in this type of analysis will not make my mistake, whether using  $V$  or other statistics, such as  $\chi^2$ . I should have calculated an expected  $V$ , based on the species' relative abundance, and then used the  $t$ -test based on the  $z$ -transformation to determine whether the calculated  $V$  departed from the expected value. It is, therefore, of value to other ecologists who use nearest-neighbour analysis, or similar methods based on contingency testing, to have the expected values of the cells.

Using the traditional notation of  $a, b, c, d$ , for the cells of the  $2 \times 2$  table, expected values for all sampling events consisting of two consecutive draws with replacement are, if the individuals are randomly arranged,  $a = 2f_x f_y$ ,  $b = f_x^2 + 2f_x h$ ,  $c = f_y^2 + 2f_y h$ , and  $d = h^2$ , where  $f_x = n_x/N$ ,  $f_y = n_y/N$ , and  $h = 1 - f_x - f_y$ .

This obviously results in an expected negative value of  $V$  that increases in magnitude as  $f_x$  and  $f_y$  increase. But it also allows a straightforward test of spatial associations comparing observed and expected values of  $V$ . If, on the other hand, nearest neighbours of the same species are classified as 'pseudospecies', one traditional method in nearest-neighbour analyses,  $f_x^2$  and  $f_y^2$  are removed from the  $b$  and  $c$  cells and added to the  $d$  cell, resulting in expected positive values of  $V$  for randomly distributed individuals.

Another alternative previously used in nearest-neighbour analyses of vegetation is to classify intraspecific nearest neighbours as 'no contacts', eliminating them from the analysis. But then each cell must be corrected by  $1 - f_x^2 - f_y^2 = m$ , which apparently has not been done, and  $a = 2f_x f_y/m$ ,  $b = 2f_x h/m$ ,  $c = 2f_y h/m$ , and  $d = h^2/m$ . Finally, I redrew (selected the next nearest neighbour of another species) when nearest neighbours were members of the same species. To my chagrin, the

## Matters Arising

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together,  $-1$  if they never do, and a value of  $0$  if the species are distributed independently<sup>5</sup>, and shows that  $V$  is negatively correlated with  $S$ . However, he seems to make no allowance for the fact that, as species richness increases,  $V$  is bound to decrease. Harris presents the argument in full.

Finally, McNaughton<sup>2</sup> draws attention to another theoretical possibility, suggested by May<sup>1</sup>, which also enhances the probability of stability in ecological systems; namely, that communities are organised into blocks of species. McNaughton suggests that the size of these blocks (or guilds) can be estimated by  $Sc$ . Unfortunately,  $Sc$  says nothing about whether the connections between species are 'blocked' and cannot measure the size of these blocks, even if they exist. Blocking depends on the arrangement of the interactions between species, and not on how many there are.

However, we agree with McNaughton that it is important to attempt to test stimulating ecological theories in the field, and

expected value of  $V$  with this approach also is negatively biased with

$$a = 2f_x f_y + f_x^2(f_y/1 - f_x) + f_y^2(f_x/1 - f_y)$$

$$b = 2f_x h + f_x^2(h/1 - f_x) + \sum_{i=1}^s [f_i^2(f_x/1 - f_i)]$$

$$c = 2f_y h + f_y^2(h/1 - f_y) + \sum_{i=1}^s [f_i^2(f_y/1 - f_i)]$$

and

$$d = h^2 - \sum_{i=1}^s [f_i^2(f_x/1 - f_i)] - \sum_{i=1}^s [f_i^2(f_y/1 - f_i)],$$

where  $f_i$  is  $n_i/N$  for each of the  $s$  species other than  $x$  and  $y$ . When I reanalysed my data, using the  $t$ -test as described above, the negative correlations I reported disappeared completely, as Harris predicted.

References 13–15, 18 and 19 in my initial Letter, as well as other sources<sup>2</sup>, can be consulted for abundant evidence justifying my assumption that the spatial arrangements of individuals in local areas may reveal the nature of interactions among them. Naturally, experimentation would be the next step.

I thank W. T. Starmer for taking me 'back to basics', showing me two of the possible ways of treating intraspecific nearest neighbours and pointing me in the direction of others.

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1. McNaughton, S. J. *Nature* **274**, 251–253 (1978).

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## Mutagenic effect of aromatic epoxy resins

ANDERSEN *ET AL.*<sup>1</sup> state that 'the demonstration of a mutagenic effect of aromatic epoxy resins (that is, those based on bisphenol acetone, BPA) indicates a genetic hazard, including a cancer risk, for humans exposed to these compounds'. We challenge the rationale for this rather definite conclusion. Epoxides as a class are reactive chemicals and in general are alkylating agents. However, in the extrapolation from direct alkylation of DNA to defining the risk of mammalian mutagenicity or carcinogenicity, other major factors must be considered, in particular the dose reaching target tissues/molecules and the importance of the detoxication or intoxication mechanisms which are only properly developed in the *in vivo* situation.

Such considerations are being included in a series of mutagenic assays to test for possible mammalian genotoxicity<sup>2–5</sup>. But at present, *in vivo* studies must be con-

sidered as giving more definitive data than work carried out *in vitro* or in non-mammalian systems.

It is surprising, therefore, when a BPA-based epoxy resin which has been tested several times for carcinogenic potential with negative results is now considered to be a potential carcinogen based on the results of a microbial mutagenicity assay (although it does indicate that *in vivo* mutagenicity studies are required). Andersen *et al.*<sup>1</sup> review some of the animal data, but we would summarise the available cancer studies as follows:

(1) Andersen *et al.* do not critically evaluate the paper by Kotin and Falk<sup>6</sup>. The latter do not provide sufficient experimental details, in particular the route and frequency of exposure, to enable any conclusion to be drawn from their work, but it can be stated that there was definitely more than one exposure to the test material.

(2) The results of Weil *et al.*<sup>7</sup> are not correctly interpreted by Andersen *et al.* They carried out two skin painting experiments. In the first, probably 30 mice were used and one papilloma was noted. In a repeat study, probably using 40 mice, no skin tumours occurred. The mortality of these mice was quite normal; there are no grounds for stating that the study was inadequate as the mice were dead in less than 24 months—the mice used by this laboratory lived their normal life span as evidenced by Weil's other data, and an average life span of about 18 months in mice is quite acceptable<sup>8</sup>.

(3) Hine *et al.*<sup>9</sup> carried out a skin painting study, not referred to by Andersen *et al.*, in which a typical BPA-based epoxy resin was tested in both mice and rabbits without any skin tumours developing. Further, reference by Andersen *et al.* to the injection site sarcoma data only must be questioned as these tumours are not generally accepted as providing any reasonable indication of carcinogenic hazard (for example, gold and hypertonic saline were shown to be carcinogenic by this route<sup>10,11</sup>).

In conclusion, the preponderance of available evidence indicates that currently used BPA-based resins present no carcinogenic hazard to man. This assessment is not changed by the evidence of Andersen *et al.*, although their findings should definitely stimulate further mutagenic studies.

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ANDERSEN *ET AL.* REPLY—Granville challenges our conclusions based on positive Ames tests with aromatic epoxy resins (AER). He claims that our conclusions are too definite and argues that tests in mammals would much better predict the risk for mutagenic and carcinogenic activity in humans. Conventional animal tests use only about 200 animals and are, therefore, rather insensitive. Only strong carcinogens will be identified by such tests<sup>1,2</sup>.

The animal tests that were made on AER in the 1950s and early 1960s are inconclusive. The tests which did not show tumours are faulty and insufficient. In several cases, exact information on the experimental details is lacking. Only about 100 mice and 50 rabbits were used<sup>3,4</sup>. The rabbit experiment was, according to the authors themselves, rather insensitive. Nevertheless, Granville claims that the experiments indicate that AER does not represent a cancer hazard, despite the experiment—albeit another one suffering from deficiencies in the description of the test conditions—in which tumours were produced by the resins<sup>5</sup>.

Thus our strong suspicions of dangerous effects of AER are in no way disproved by existing animal tests, nor will any new animal carcinogenicity test be able to give definite proof for non-carcinogenicity. It is our opinion that the doubt which might exist about the mutagenic or carcinogenic activity of a chemical substance should be used for the benefit of people who will come in contact with the substance. The only clear proof that AER is not dangerous to humans could come from experiments proving that AER cannot reach the relevant target molecules, namely DNA. As long as these experiments have not been made, AERs must be considered as mutagens and carcinogens.

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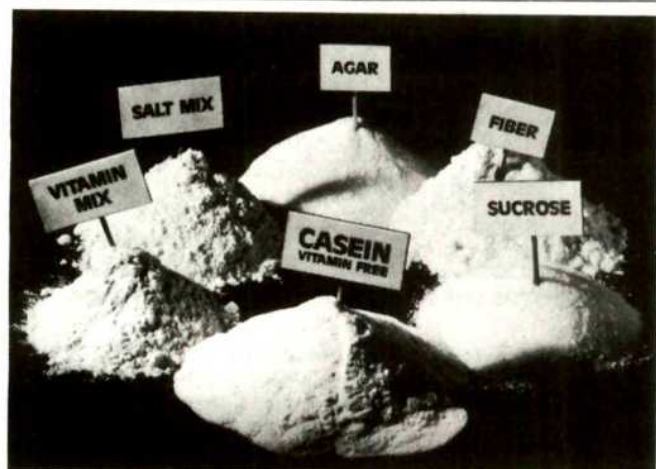
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# reviews

## Exciting endocrinology

J. A. Parsons

*Pioneers in Neuroendocrinology*, Vol. II. Edited by J. Meites, B. T. Donovan and S. M. McCann. (Plenum: New York and London, 1978.) £20.48.

THIS fascinating collection of micro-autobiographies will delight those who already know that scientists as a group are neither more nor less often inspired, neither more perfect nor more perverse than others who make a living by their wits. It will also be received with joy by those who doubt the second of both these pairs of propositions. There is enough in it to reinforce almost any prejudice and material for many an after-dinner story.

Two quotations may whet the appetite and show the potential of the book in arguments for or against the 'Two Culture' hypothesis.

"Harmony in science respects the same type of stringent rules that can be found in Bach's polyphony . . . It really does not matter, for the progress of science, whether you are personally exploiting one of your previous findings and working on the next discovery, or whether the next step is taken by others who have picked up your idea. Some people feel this is stealing from you. In my opinion, it is the best compliment one scientist can pay another".

"My one application to Jefferson Medical College was rejected, to my parents' chagrin but to my delight, and for the next two years I worked as a professional musician in the Philadelphia area".

Inevitably in a book of this type, the contrast of personal philosophies is as remarkable as the difference in literary styles, but most contributions are penetratingly self-critical and many are full of humour.

"Du Vigneaud once told me that in the isolation of oxytocin, the most efficient purification step had been that of separating the pituitary from the cow".

Also, at a time when the number of young people of exceptional ability who choose scientific research as a career seems to be falling, one value of such a collection of honest attempts 'to tell it like it is' may be to provide an insight into the challenges and rewards of this way of life, far more vivid than any description from a careers officer.

Contributions to this volume were of course only invited from those whose work has succeeded, in the sense of making important contributions to a

relatively new branch of endocrinology. Another way in which its publication can be of general value is therefore by allowing some comparison of research careers and of the contributions made by different types of funding.

As one would expect in our era, the overwhelming majority of the work has received state support. The total of 24 contributors is not a large statistical sample, but it is noteworthy that 20 worked with grants in a university environment. Of the remainder, two were on the staffs of privately endowed research institutes (though they, too, received much state funding), two carried out some of their work in government research institutes, and one was supported principally by the US Veterans Administration.

As it can be predicted with some confidence that during the next decade neuroendocrinology will make major contributions to drug treatment of disease, it is again of some interest that 14 of the contributors had their formal training in physiology and/or anatomy, four in zoology, two in psychology and only two in pharmacology. Only five mention industrial support for some of their work. No less than 12 of the authors were trained as physicians before specialising in a basic science, a career pattern which has now become very rare in the UK.

The editors evidently had no distaste for controversy and several spectacular old battles receive an airing, with much detail of intellectual arguments and strategy and at least one allegation of foul play. The history of science in the making is clearly fraught with the same problems as those encountered in histories of the more distant past. As usual, the details of such accounts are of greatest interest to the participants and those responsible for funding them, but it seems worth drawing attention to one issue of general significance.

Major scientific journals exercise great power, and the forces of competition tend to ensure that their editors exercise this responsibly in maintaining the quality of work accepted. However, an incident which is discussed at some length raises the question whether editors are equally conscious of the responsibility incurred in rejecting a paper. Many will agree that the regret-

table practice of anonymous rejection by the use of a supply of forms is too common; justice and the public interest seem to require that dismissal of any carefully presented paper should be based on equally serious scientific assessment. Not only may ill-considered rejection delay general awareness of work later recognised as important, perhaps by several years if authors are diffident; as illustrated by the incident discussed, still worse may ensue if the rejection is based on comments of a single carelessly-chosen reviewer, who has thus received privileged communication of the results (most dangerous of all if he is a competitor). Justice is usually done in the end—but at what cost?

Quite apart from the human interest, the book is worth reading for its excellent science. For example, there are some of the clearest discussions one could wish of the (unfinished) list of frustrations encountered in trying to isolate the Corticotrophin Releasing Factor. There are also valuable insights into the role of the hypophysial portal circulation, presented notably by Bogdanove and Halász. Incidentally, although its relevance is not directly discussed, this evidence for highly directional transfer rather than simple diffusion of hypothalamic factors should surely lead to an armistice in the old battle over functional significance of the portal vessels. (The argument is here pursued unrelentingly by Lord Zuckerman himself, while Harris' perceptions are ably defended by Dr Donovan.)

In short, the editors are much to be congratulated for their enterprise and for showing clearly how much further excitement neuroendocrinology is likely to provide in the coming years. Perhaps we can look forward to another volume when such important issues are resolved as the structures of CRF, GRF and MIF, the role of prolactin in the origin and development of mammary tumours (interestingly discussed by Meites), and the probable physiological role of centrally acting peptides affecting memory and behaviour (excellently reviewed by de Wied). □

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## Planetary atmospheres

*Theory of Planetary Atmospheres: An Introduction to their Physics and Chemistry.* By J. W. Chamberlain. Pp. 330. (Academic: New York, San Francisco and London, 1978.) £19.15.

It is a brave person who considers writing a textbook on planetary atmospheres at this time when there are so many space missions to the planets rapidly advancing our knowledge of these bodies. One procedure is to concentrate on basic processes and then use the current planetary observations to illustrate the mechanisms involved. Basically, this is the approach adopted by Professor Chamberlain whose valuable textbook is primarily concerned with the physical and chemical properties of planetary atmospheres. The author has made good of our effluent society, which in the past few years has produced detailed investigations of photochemical processes related to the potential climatic impact of variations in the Earth's ozone layers. This has allowed the discussions to be placed on a moderately firm foundation before their application to the planets.

The book covers approximately 300 pages of text supplemented by some useful appendices. Each of the seven chapters has a valuable list of references, that may be used to extract more detailed analyses of the work presented. In addition, there are problems at the end of chapters that make the text useful as a teaching aid for the rapidly increasing number of courses in planetary studies at universities throughout the world.

The introductory chapter discusses the basic physical concepts and provides the reader with a good background to the vertical structure of the Earth's atmosphere, together with an indication of the differences of structures of the other planetary atmospheres. I thought that the comments on p40 regarding the initial interpretation of the Pioneer 10/11 results for the structure of the upper atmosphere were unnecessary. The problem first identified when reducing these measurements has been resolved. Furthermore, the Voyager observations had now added further important information that indicates interesting structure in the upper Jovian atmosphere which now means that part of Figure 1.19 is out of date. I had hoped that Professor Chamberlain would not have introduced the term "dust" on p38 when discussing the ultraviolet absorbing layer created from photochemical products thought to reside above the visible clouds. This is an unfortunate term, misused for too long.

The introduction to atmospheric motions is well presented, although some aspects of the planetary examples are a little sketchy. In particular the reader still would have no idea why Jupiter (and Saturn too) have cloud bands from the discussions presented here. With many aspects of the Jovian dynamics still to be resolved, it would probably have been better to have shown how spacecraft measurements that could help resolve the controversial issues that currently exist.

The next two chapters are concerned with the chemistry and dynamics of the Earth's stratosphere and planetary astronomy where the basic concepts of radiative transfer are discussed. Professor Chamberlain is well known for his work in these areas, and the work is well presented. I still maintain, from a personal viewpoint, that the classical Chandrasekhar approach to radiative transfer is not the easiest way to introduce the subject to the reader. How-

ever, the references at the end of the chapter contain papers with other approaches, so the reader can decide this matter for himself.

The remaining chapters are concerned with ionospheres, airglows and aeronomy and the stability of planetary atmospheres. This indicates the breath of the discussions which with a good mathematical description form the basis of a useful book. I personally consider this volume a valuable addition to the subject. I sincerely hope, therefore, that the publishers will have the wisdom to produce an inexpensive paperback version so that students attending courses in this rapidly growing subject will be able to purchase a copy of their own.

Garry E. Hunt

*Garry E. Hunt is Head of the Laboratory for Planetary Atmospheres, Department of Physics and Astronomy, University College, London, UK.*

## Guide for Darwin scholars

*Charles Darwin: A Companion.* By R. B. Freeman. Pp. 309. (Dawson: Folkestone, UK; Archon Books/Shoe String Press: Hamden, Connecticut, 1979.) £12.50; \$27.50.

RICHARD FREEMAN is rightly held in high regard for his meticulous labours on the bibliography of Charles Darwin's writings. His equally significant work *Charles Darwin: A Companion* is likely to prove indispensable to Darwin scholars. Interest in Darwin continues to grow, as continued exposure on the media attests and the steady flow of learned publications sustains. Recent discoveries are deftly and economically incorporated with notable conciseness.

There is a clear need for an enumeration of securely determined facts about Darwin: about his family from the sixteenth century record in Lincolnshire to his grandchildren; about the servants, dogs and horses as well as those of their close friends; about the names and ever baffling nicknames of their ramifying and closely intermarrying relatives; and about the Hookers, Huxleys, Henslows and Wedgwoods.

The *Companion* is an alphabetical list of names; wives are listed under their maiden name with a cross reference to their husband. All cross-references are cunningly devised to encourage wandering as should always be the case in a good work of reference. Quotations from Darwin enliven the identity and relevance of each name so that the book becomes a truly

fascinating anthology of quotations as well as a detailed guide to the formidable bibliography. The forty-one pages of material devoted to Charles Darwin required 25 sub-headings listed on page 71. The sub-headings are set in an insufficiently bold type and it would have been helpful to the reader to carry the sub-heading at the top of the relevant page. Particularly worthy of note are the "few quotations to give indications of CD's character" (pages 71-2) which include new discoveries amongst old friends. In his introduction Freeman quotes Darwin's letter to Huxley from Ilkley in November 1859 (LLii281): "The difficulty is to know what to trust." when making a compilation from various sources. Richard Freeman, an accurate compiler with a discerning eye for the unusual, is sadly let down by careless transcriptions in the printed texts. Both Francis Darwin and his sister Henrietta placed unjustified faith in the transcriptions they published. Hence, we read instead of Moscheles as teacher of pianoforte to their mother the name Maschelas, and Henrietta transcribes the name of Darwin's amanuensis and companion Syms Covington as Conington (Emma Darwin ii 19). It was a more recent misreading by a dealer in manuscript letters which resulted in the entry on page 167 for Hoskins an untraceable botanist. The original of this letter to Henslow has since come to light and reveals that CD wrote Hooker, indeed a botanist and in 1845 an unsuccessful candidate for the Chair of Botany at Edinburgh.

Sydney Smith

*Sydney Smith is Emeritus Fellow of St Catharine's College, Cambridge, UK.*

## Theory of conditioning

*Classical Conditioning and Operant Conditioning: A Response Pattern Analysis.* By W. W. Henton and I. H. Iversen. Pp. 355. (Springer: New York, Heidelberg and Berlin, 1978.) DM 54; \$29.70.

THE argument of this book is that the phenomena of conditioning can best be understood by observing behaviour in all possible detail, and describing the effects of contingencies between stimuli, rewards and responses on everything the subject is doing. The authors build up an impressive, if partisan, case for this "response pattern" theory and for the "multiple response" experimental technique which it implies. Along with these latter, however, they bring us a metatheoretical commitment to a hard-line Skinnerism, according to which even the editorial policy of the *Journal of the Experimental Analysis of Behavior* is dangerously revisionist. Any attempt to use behaviour as an index of an underlying state, physiological or mental, is consistently condemned as "phrenology".

This package of experimental method, theory and ideology is applied to five problems: classical conditioning against an instrumental baseline (conditioned suppression and its variants); concurrent operant performances; multiple schedules of reinforcement; collateral (adjunctive) behaviours; and concurrent classical conditioning. In each area the authors produce original experimental ideas and have provocative remarks to make about currently dominant theories, and they repeatedly show that if the attempt is made to record all an animal's behaviour, regularities of response sequencing can be found to underlie effects of reinforcement schedules that have mainly been analysed at a more molar level in the past.

Although Henton and Iversen break away from recording a single "arbitrary operant" response, they still use arbitrary subjects (rat, pigeon, monkey) in wholly artificial, stereotyped learning situations. But it is a little unfair to criticise them for faults they share with most of operant psychology. More seriously, they entirely ignore the regulatory aspect of instrumental responding, which must constrain the subject towards that "averaging over minutes, hours or days" which the authors condemn. In fact they never ask themselves either what the behaviour they observe so microscopically, or the conditioning they are trying to explain, might be for. I cannot help feeling that this blindspot, which greatly weakens

the authors' argument, is partly due to their ideological position.

Minor niggles include the lack of an author index (exacerbated by having separate bibliographies for each chapter), a high misprint rate, a slightly petulant tone savouring of "Reply to Reviewer B" in places, and occasional lapses from English idiom, which, though never a threat to understanding and also forgivable from Iversen, ought to have been caught by his publisher or co-author. There are two serious omissions from the point of view of the reader's convenience. First, the authors miss the opportunity to establish a consistent way of presenting multiresponse data. No book containing detailed accounts of 31 experiments can make easy reading, but repeated changes in

diagram conventions make things harder than they need be. Secondly, we could have done with summary tables, for each chapter or the book as a whole, giving the main procedures and results of all the experiments. As it is, there isn't even a concluding chapter to review and summarise the argument. Nevertheless, the book deserves a welcome, partly for giving an integrated account of an extended research programme, but mainly as an empirical challenge to some widespread current generalisations about conditioning.

S. E. G. Lea

*S. E. G. Lea is Lecturer in Psychology at the University of Exeter, UK.*

## Sensory integration

*Handbook of Behavioral Neurobiology.* Vol. 1: Sensory Integration. Edited by R. B. Masterton. Pp. 579. (Plenum: New York and London, 1978.) £24.88.

IN the words of the publisher *The Handbook of Behavioral Neurobiology* will provide "a critical systematic enquiry into those aspects of neuroscience having the most direct and immediate bearing on overt behaviour." The targets for this first volume are "practising neuroscientists looking for a concise and authoritative treatment of developments outside their particular specialisation, and students who need an overview of the persistent and current problems surrounding the relation of the perceptual systems to behaviour". The eighteen authors were asked to "sacrifice comprehensiveness for illumination". Without exception they have complied with editorial pressure and in so doing have produced a surprisingly well integrated book which should achieve its aims.

The ten chapters dealing with the individual sensory systems form the core of the book. Not surprisingly the visual system claims more space than any of the others, but it is closely followed by the vestibular and auditory systems. Olfaction, in some ways the most interesting of the senses, brings up the rear with less than half the space earned by the gustatory and somatosensory systems. The reader, be he a practising neuroscientist or a student, will probably first turn to one of these chapters; and he will not be disappointed. Without exception the authors have provided illumination and not always at the expense of comprehensiveness.

It would be a pity, however, if the first three integrating chapters escaped attention. Erickson who writes chapter three prefaces it with a quotation from Poincaré: "Science is built up with facts as a house is with stones. But a collection of facts is no more a science than a heap of stones is a house". These first three chapters provide the reader with plans for a house. Erickson himself argues that our knowledge of sensory processes will be constrained "if we resist seeing beyond the idiosyncracies of each system". He then moves towards a general theory of sensory neural function. In the second chapter, Cunningham and Murphy explore the ontogeny of sensory systems and ask whether their developing structure and function are wholly genetically determined. Despite the weight of the present evidence they think not, and predict that future work using anatomical and physiological techniques which can detect the subtleties of sensory perception will show that the contribution of nurture to sensory development is the rule rather than the exception.

Finally the editor's opening chapter surveys the evolutionary history of the six sensory systems. For each he provides a useful common plan for all vertebrates and then briefly discusses any striking variations. He concludes each section with a discussion of the notable changes which have probably occurred in the evolution of the human sensory system in question.

These three chapters provide a good introduction to a useful book which sets a high standard for those which are to follow.

J. R. Symons

*J. R. Symons is Professor of Psychology at the University of Aberdeen, UK.*

## Vertebrate ovary

*The Vertebrate Ovary: Comparative Biology and Evolution.* Edited by R. E. Jones. Pp. 853. (Plenum: New York and London, 1978.) £43.78.

PUBLICATION of another volume (over 800 pages) on the ovary highlights the expansion of research in this aspect of reproductive biology. Consisting of 24 reviews by leading scientists predominantly from the USA the work is mostly comparative in nature. This is exemplified at the beginning and end of the book by chapters on the origin and segregation of primordial germ cells, in which the anuran germ line receives special attention, and on the evolution of the vertebrate ovary. Morphological differentiation of the ovary is also considered from a comparative viewpoint, but biochemical and genetic factors that influence gonadal differentiation are treated only briefly.

The follicle is reviewed extensively: its genesis, morphology and endocrine function; the hormonal control of its growth and maturation; and the production of follicular fluid. The non-atretic follicle is seen as developing in a predominantly oestrogenic micro-environment that influences follicular growth; processes that result in atresia, and the repercussion of this phenomenon on ovarian function, are examined separately.

Understandably, formation of oogonia, maturation of the oocyte and ovulation receive much attention. Most contributors dealing with mechanistic aspects of ovarian physiology summarise their ideas in the form of hypothesis. This is seen in the description of ovulation, and of oocyte maturation in amphibians, in which the elaborate biochemical events induced by steroids that lead to the formation of the meiotic spindle are examined. Work on this latter aspect contrasts with experiments in mammals which have focused rather on the maturation-inducing action of luteinising hormone and on how the resumption of meiosis is prevented by granulosa cells and follicular fluid inhibitor.

Compared with its close contemporary, *The Ovary* (edited by Lord Zuckerman and B. J. Weir; second edition; Academic: London and New York; three volumes; total price £77.70), it is inevitable that this new book contains chapters on similar topics. However, interpretations by different experts frequently result in essays that are complementary rather than merely repetitious. Valuable additions include reviews of ovarian vasculature, with a description of techniques of blood flow measurement and of the effect of

humoral agents, ovarian innervation, and the fascinating fields of follicular selection and vertebrate fecundity. Discussion of the fate of the ruptured follicle is less satisfactory, as reference to the evolutionary significance of the corpus luteum and the comparative aspects of its structure and function are found dispersed among several reviews. The lack of chapters on ovarian pathology and the influence of external factors on ovarian function is attributed to the restrictions of space, but for a book of this quality the reproduction of photomicrographs deserves a better standard.

A selected sentence from a paper

by Bern (1972) reflects the editor's bias: "we comparative biologists have an aim—the aim to reconstruct from extant species a picture of the evolution of the system in which we are interested". The picture proves complex, frequently displaying diversity rather than conformity. Nonetheless the book should prove valuable for the research scientist, clinician and teacher; and the nature of unanswered questions should stimulate enquiry.

R. B. Heap

R. B. Heap is Head of the Department of Physiology at the ARC Institute of Animal Physiology, Babraham, Cambridge, UK.

## Climatic impact on organic evolution

*Climate and Evolution.* By Ronald Pearson. Pp. 274. (Academic: London, New York and San Francisco, 1979.) £14.

THIS is an ambitious work that must be taken seriously by anyone interested in palaeoclimatology and biostratigraphy. Such a study, in order to fulfil its title, requires a multi-disciplinary approach to a vast literature and is more than an ordinary mortal could achieve. Pearson, however, is mortal enough, so it is worth seeing where he succeeds and where the book falls short.

The book is organised so that chapters 1–5 are general and 6–11 historical. The introduction concerns evolution only and is brief but to the point. Chapters 2, "A brief history of historical climatology", and 3, "More recent knowledge of climatic change", review the principal hypotheses for climatic change but primarily for changes evident in Quaternary history. This is to some extent balanced by chapter 5, "Long term considerations", with chapter 4, "Geomagnetic considerations", thrown in for good measure. The result is a very brief resume of the mechanisms for climatic change abstracted from many leading authorities in these fields. The remaining chapters are chronological: (6) Palaeozoic; (7) Mesozoic; (8) Tertiary; (9) Quaternary; (10) Late Weichselian and Flandrian; and (11) Climate and [human] history. A consolidated list of more than 750 references and a fairly good index, complete the book.

The author's own viewpoint is evident in the balance of the work. His own publications listed concern Quaternary Coleoptera; and the whole discus-

sion is Quaternary-orientated, so that earlier and possibly more radical climatic and evolutionary changes are increasingly obscured through the mists of time. Precambrian history is accorded token mention and the first metazoan (Ediacaran) fauna (surely of prime significance in such a study) receives only a passing reference without definite mention. In the same chapter discussion of climatic change on the megascale is minimal. For example, the role of atmospheric CO<sub>2</sub> is hardly discussed and the possibility of the first half of the Earth's history being climatically hotter than later because of this and other factors, is not considered. Inevitably too, the consideration of Pre-Quaternary history depends on more stratigraphical knowledge than is easy to acquire for the purposes of such a work.

An alternative approach might have been more satisfactory, namely, to review organic evolution and first identify the developments that need explanation and then to compare climatic and other mechanisms in each case against a critical assessment of the chronological precision of the data brought to bear. And here is a difficulty: inevitably the many chronometric age estimates quoted tend to be taken at their numerical face value, and it would require much research to do otherwise.

In conclusion, this work, except for parts of the Quaternary record, does not provide a critical assessment of the climatic impact on organic evolution but it does a most valuable service in bringing together and abstracting a variety of relevant literature and the wide range of ideas necessary as a preliminary. Most specialists will find something here to challenge their thinking.

W. B. Harland

W. B. Harland is Reader in Tectonic Geology at the University of Cambridge, UK.



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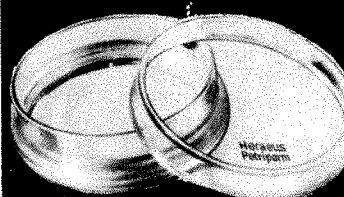
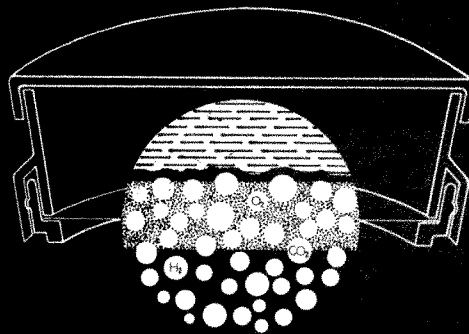
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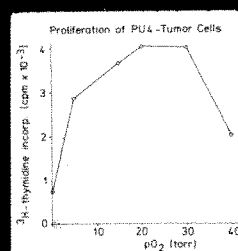
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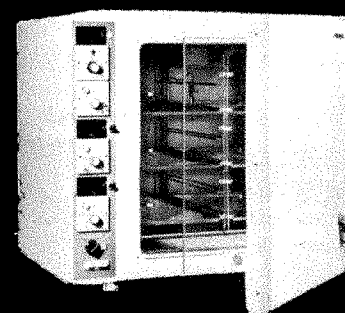
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# obituary

## Sir Edward Salisbury

SIR EDWARD JAMES SALISBURY, CBE, FRS, who died on 10 November 1978 at the age of 92, was one of the best known British botanists of his generation.

He was born on 16 April 1886 at Limbrick Hall, Hertfordshire, his father, J. Wright Salisbury, being a member of a distinguished family long resident in the area. He was educated at University College School and then at University College, London, which he entered as an undergraduate in 1905, graduating with an honours degree in botany in 1908. He stayed on as a research student, from 1910 onwards as Quain Student; moved to East London College in 1914 as senior lecturer in botany, and returned to University College as lecturer in 1918. In 1924 he was made university reader in plant ecology, and succeeded F. W. Oliver as Quain Professor in 1929, retaining this post until he was appointed director of the Royal Botanic Gardens, Kew, in 1943. He retired from the directorship in 1956 at the age of 70. He was elected Fellow of the Royal Society in 1933, was awarded its Royal Medal in 1945, and served as Biological Secretary from 1945 until 1955. He was made CBE in 1939 and was knighted in 1946.

Salisbury showed an interest in plants at quite an early age. By his 15th birthday, when one of his presents was a copy of Hooker's *Student's Flora*, he had a garden plot in which wild plants he had collected were labelled with their Latin names, had formed a private herbarium and could identify most of the flowering plants of Hertfordshire. He went to University College during an important period of change in attitude towards the scientific study of plants.

The professor of botany was F. W. Oliver, and A. G. Tansley was a lecturer on his staff from 1893 until his return to Cambridge in 1907. Both had been much influenced in their research and teaching by the striking advances in palaeobotany of the few previous decades. Just before the turn of the century, however, Oliver embarked on his studies of coastal vegetation and Tansley became deeply interested in the 'ecological' and physiological aspects of plant geography as expounded by Warming and Schimper respectively. They had been increasingly dissatis-

fied with the almost exclusive attention to comparative morphology and anatomy which still characterized much botanical teaching and welcomed the ecological emphasis on the plant as a functional whole.

Salisbury was immediately attracted by Oliver's lectures and especially by his field excursions to the north Norfolk coast and elsewhere. After graduation he elected to work with Oliver and two joint papers appeared in 1913, one being a lengthy general account of the ecology of Blakeney Point. Meanwhile his ecological interests were extending to problems of inland vegetation and especially of woodlands, and his now classic studies of variations in the woodland light climate with season and with stage in the coppice-cycle, and their effects on the ground flora, soon attracted attention. He was already laying the foundations of his reputation as a leading academic botanist, and his appointment to the Quain Chair in 1924 greatly increased his influence.

This was early exerted through the series of text-books written in collaboration with F. E. Fritsch. *An Introduction to the Study of Plants* was published in 1914 by G. Bell & Sons and was immediately successful in encouraging the consideration of plants as living and functioning wholes rather than as assemblages of organs, tissues and cells. The last of the famous 'Fritsch & Salisbury' series was *Plant Form and Function* (1938).

While still a research student Salisbury was invited to join the British Vegetation Committee, set up in 1904 to coordinate current work on the survey and study of British vegetation and converted in 1913 into the British Ecological Society. Salisbury was a founder member of the Society and was invited to join its council and to become hon. secretary in 1915, an office he held until 1931. He was elected president for the two years 1929-30.

His presidential address, 'The biological equipment of species in relation to competition' (1929), reveals very clearly how far British ecology, with its primary aim of understanding why plants of some species but not of others grow in a given area, had come to differ from continental ecology with its largely floristic interest and its emphasis on the description and classification of plant communities. Certainly Salisbury was relatively little interested in plant communities as such. His main

concern throughout his life was with those features of individual plant species that were most relevant to survival in a given environment and against given competitors, and in particular those that could readily be assessed quantitatively. The best of his books—*The Reproductive Capacity of Plants* (1942) and *Weeds and Aliens* (1961)—and large numbers of papers in scientific journals, examine the survival-value of features of seed-production, or of vegetation multiplication and spread, in species of different habitats. They are packed with original data, especially of counts and weights of seeds produced by various species in various circumstances. Eleven of the fifteen papers published during the final ten years of his life are of this kind and the last of all, appearing in the year of his death at 92, included a table of estimated annual production of seeds by 49 different weed species 'based upon my own observations from random samples.'

It is relevant to reflect that Professor J. L. Harper's important book, *The Reproductive Biology of Plants*, is in many ways a continuation of *The Reproductive Capacity of Plants*, with a successful elucidation of many points left unexplained by Salisbury but with a considerable widening of scope and a far greater emphasis on experimentation. Characteristically, however, Salisbury had perceived many of what were still the outstanding problems in 1977.

Early in his career Salisbury became a member of the Royal Horticultural Society and was for many years a vice-president. He took great delight in gardening and after his marriage developed a notably attractive and botanically very interesting garden at his home Willow Pool, Radlett. *The Living Garden, or The How and Why of Garden Life*, a skilful popular exposition of the scientific basis of horticulture, appeared in 1945. It was immensely successful and led to the award of the Veitch Memorial Gold Medal of the RHS that same year, and he later received the society's Victoria Medal of Honour. His appointment to Kew was a fitting climax to his career.

Clear-headed, self-confident, determined and articulate, Salisbury was very much a committee man and served on innumerable councils, committees, delegacies and visiting groups. He was governor of several colleges and research institutions and a successful

chairman of many important public bodies. He was short of stature, lively and friendly in manner, but perhaps rather too fond of indulging his delight in lengthy exposition. He was an able administrator. In the later stages of his career, however, he spent much time on his outside commitments.

In 1917 Salisbury married Mabel Elwin-Coles, who died in 1956 after a long illness. There were no children.

A. R. Clapham

## C. T. Rajagopal

PROFESSOR Cadambathur Tiruvenkatacharya Rajagopal, a mathematician of considerable standing in India, and solely responsible for the survival in Madras of the Ramanujan Institute, died on 25 April 1978. Even more, perhaps, than his numerous original contributions to mathematics and its history (which were substantial by any standards), it is his devotion to the cause of mathematics in India and to the survival of the Institute (which he served in various capacities from 1951 to 1971) which made him a unique figure in an important period of the history of Indian science, deserving to be remembered by future generations of Indian scientists.

C. T. Rajagopal was born on 8 September 1903. His father, Cadambathur Tiruvenkatacharya, was in the judicial service of the Madras Presidency (now Tamil Nadu). His early education was in Madras; he took his Master's degree in the Madras Presidency College (where many of the most distinguished scientists of India, including Sir C. V. Raman, have been students). There he soon came under the influence of Professor K. Ananda Rau, himself a mathematician of great distinction, who had been G. H. Hardy's student and Ramanujan's contemporary in Cambridge. Ananda Rau is well known and remembered for his valuable contributions to the theory of Tauberian theorems, function-theory and the theory of Dirichlet series; and his tastes and interests were decisive for the orientation of Rajagopal's future scientific career.

After graduating from the Presidency College, Rajagopal joined the Madras Christian College as a lecturer. In 1951, T. Vijayaraghavan, on his appointment as director of the Ramanujan Institute, invited him to join its faculty; the story of Rajagopal coincides with that of the Institute for the following twenty-five years.

The Ramanujan Institute was founded in 1951 as a private institution

by the late Sir Alagappa Chettiar, a noted philanthropist of South India, as 'a small remembrance of a great man (Srinivasa Ramanujan). Its first director, T. Vijayaraghavan, was perhaps the most talented among G. H. Hardy's former students; he died at a comparatively early age in 1955; Rajagopal took over the directorship from him. Already at that time the financial status of the Institute seemed shaky, since Alagappa Chettiar's fortune was melting away; T. Vijayaraghavan's family was left unprovided for, and an appeal to the Prime Minister (the late Jawaharlal Nehru) had to be made in order to rescue them from utter poverty.

In April 1957, when Alagappa Chettiar died, the fate of the Institute hung in the balance; Rajagopal wrote to one of us (S.C.) that the Institute 'will cease to exist on the first of next month,' whereupon the addressee wrote to the Prime Minister, explaining the origin of the Institute and the seriousness of its condition. Nehru's prompt answer was refreshing: 'Even if you had not put in your strong recommendation in favour of the Ramanujan Institute of Mathematics, I would not have liked anything to happen which put an end to it. Now that you have also written to me on this subject, I shall keep in touch with this matter and I think I can assure you that the Institute will be carried on.'

And it was; but haltingly and precariously for the next twelve years. The responsibility for the Institute was divided between the U.G.C. (the federal University Grants Commission) and a reluctant University of Madras. There is no doubt that the Institute would not have survived had it not been for Rajagopal's continuing year after year with an uncertain appointment and often as the sole 'permanent' member of the Institute. In 1963 the future of the Institute and Rajagopal's own means of survival were so much in doubt that he wrote us in the following terms: 'In twenty-one years of service as a teacher (of which the first year was spent in Annamalai University and the rest in Madras Christian College), my salary rose from Rs.100 p.m. to about Rs.240 p.m. and earned for me a provident fund of nearly Rs.8,000. In the next twelve years of my service, in the Ramanujan Institute, my salary scale was Rs.500 - 50 - 800 until I was made a professor with effect from 1st March 1962 on Rs.850 p.m. in the present Madras University scale of Rs.800 - 50 - 1,250. However, my service in the Institute has left me with no savings and no retirement benefits. Thus, after a professional life of thirty and odd years, I find myself without the means to live in complete independence . . . '.

But Rajagopal did continue to serve the Institute for the following six years, and at long last, in August 1967, the Ramanujan Institute was finally adopted by the University of Madras, and in July 1969 a new director was appointed. Its subsequent fortunes do not concern us here; but this left Rajagopal with no pension; not a single *naya paisa* (the new half-penny), as he himself wrote; the undersigned, singly or jointly, must have written dozens of letters to various authorities during the years 1963-1978 to secure for him a modest stipend of Rs.500 p.m.

Despite such administrative and personal worries and frustrations, and without ever an opportunity for visiting any centre of mathematics in Europe or the United States, Rajagopal maintained an unabated output of competent, worthwhile mathematical research, well appreciated by co-workers in his favourite fields, chiefly Tauberian theorems and entire functions. In the latter part of his life, he became actively interested in the history of medieval Indian mathematics, to which he contributed a number of important papers, partly in collaboration with others; the last one, a joint article with M. S. Rangachari, appeared only a few weeks before Rajagopal's death, in C. Truesdell's well-known *Archive for History of Exact Sciences*, vol. 18, pp. 89-102, 1978. There it is shown that a number of power-series expansions for trigonometric and inverse trigonometric functions, discovered in the 17th century by Gregory, Newton and Leibniz (and independently, about the same time, by the Japanese Seki), had been known to Kerala mathematicians more than a century before.

Professor C. T. Rajagopal is survived by his wife, Mrs. Rukmini Rajagopal (now living in straitened circumstances, it must be pointed out, due to the lack of provision for a pension fund at the Ramanujan Institute). Tragically, Rajagopal's death occurred just as a grant had been approved to support the continuation of his and M. S. Rangachari's historical investigations, which were to appear eventually as a monograph on Kerala mathematics.

India may well have produced mathematicians of greater versatility and depth than C. T. Rajagopal, and will, one hopes, produce more such in the future; but none has served the cause of mathematics more selflessly nor with greater devotion. As both of us happen to have been personally associated, for many years, with this modest and talented man, we find it fitting that his long years of service be placed on record.

S. Chandrasekhar  
André Weil



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## APPOINTMENTS VACANT

### UNIVERSITY OF SYDNEY RESEARCH FELLOW IN FUSION PLASMA PHYSICS

Applications are invited for a Postdoctoral position in fusion-orientated plasma physics. The appointment will be for two years and is funded by NERDDC. The appointee will work with the research tokamak under construction in the School of Physics and will be mainly responsible for the operation and development of diagnostic techniques, particularly laser scattering. The group possesses high power ruby and CO<sub>2</sub> lasers and a multichannel digital data acquisition system.

Salary will be in the range \$A15,786 to \$20,737 per annum. It is desired to fill the position early in July 1979.

Applications, including curriculum vitae, list of publications and names of three referees by **June 15, 1979** to Associate Professor J. Lehané, School of Physics, University of Sydney, NSW 2006, Australia, from whom further information available.

960(A)

### UNIVERSITY COLLEGE OF SWAZILAND

Applications are invited for the post of  
**LECTURER**

in the

**DEPARTMENT OF BIOLOGY**

Candidates should possess at least an M.Sc. in Biology, although a Ph.D. would be desirable. Strong preference will be given to candidates who have had experience at the University level. A broad background of preparation will be an advantage. The appointee will be required to conduct lectures and laboratory periods in Biology, presently in years 1 and 2 of the B.Sc. course, Part I; and to perform standard duties in the day-to-day running of the Department's programme. Part II studies are scheduled to begin in August 1980, with the introduction of six three-credit courses in third year Biology, followed by six more in the following year. Preparation for this extension of programme will be included in the duties pertaining to the post.

Salary scale: Lecturer I, E5,940 to E7,860 per annum (£1 sterling=E1.75). The British Government may supplement salary by £1,650 to £2,184 per annum (sterling) for married appointee or £408 to £960 per annum (sterling) for single appointee (reviewed annually and normally free from tax) and provide children's education allowances and holiday visit passages. Family passages; short-term contracts of 2-4 years; 25% gratuity in lieu of superannuation for first 2 years and 27½% for second 2 years; 10% inducement allowance for those not qualifying for supplementation from other sources; various allowances.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to Registrar, University College of Swaziland, Private Bag, Kwaluseni, Swaziland, by July 10, 1979.

Applicants resident in the U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address.

858(A)

### UNIVERSITY OF WESTERN AUSTRALIA Perth LECTURESHIP IN INORGANIC CHEMISTRY

Applications are invited for appointment to the above position in the Department of Physical and Inorganic Chemistry. Preference will be given to applicants with experience in preparative and/or organometallic chemistry.

The appointee will be expected to share in the teaching of general inorganic chemistry and of organometallic chemistry at the undergraduate level, to lecture in his field of specialisation at senior undergraduate and at postgraduate levels and to carry out research for which time and good facilities are available.

The School of Chemistry occupies a well equipped building with machine, electronic and glass-blowing workshops. A wide range of equipment for physical measurements is available, and the University has good computing facilities. The staff of the Department of Physical and Inorganic Chemistry consists of fifteen members and there are 35 postgraduate and postdoctoral research workers.

The current salary range for a Lecturer is: \$A15,786 to \$A20,737 per annum. Benefits include superannuation similar to F.S.S.U., fares to Perth for appointee and dependents family, removal allowance, study leave and long service leave and housing loan scheme.

Applications in duplicate stating full personal particulars, qualifications and experience should reach the Staffing Officer, University of Western Australia, Nedlands, Western Australia, 6009, by **June 30, 1979**. Candidates should request three referees to write immediately to the Staffing Officer.

961(A)

### PLANT BREEDING INSTITUTE

Maris Lane, Trumpington,  
Cambridge CB2 2LQ

**SCIENTIFIC OFFICER/  
HIGHER  
SCIENTIFIC OFFICER**

**FORAGE, OIL AND POTATOES  
DEPARTMENT**

A pathologist is required in the Forage, Oils and Potatoes Department to work on diseases of oilseed rape. This work is to be carried out in close co-operation with the oilseed rape breeder, Dr K. F. Thompson, and would involve developing mature-plant and seedling tests for resistance to stem canker, *Phoma lingam*, and light leaf spot, *Pyrenopeziza brassicae*. The work on canker would necessitate searching for new sources of resistance. The officer would also undertake glasshouse screening tests for resistance to clubroot, *Plasmodiophora brassicae*, and eventually possibly for *Alternaria*, *Botrytis* and *Peronospora*. A knowledge and interest in genetics would be useful as investigations on the inheritance of resistance to *Phoma* and *Pyrenopeziza* are planned.

Candidates should have a 1st or Upper 2nd Class Honours Degree specialising in Plant Pathology or equivalent; experience of Brassicas would be of an advantage.

The appointment will be as Higher Scientific Officer (£4,101 to £5,448 per annum) or Scientific Officer (£2,839 to £4,415 per annum) according to qualifications and experience. At least two years relevant postgraduate research experience is required for appointment as Higher Scientific Officer. Non-contributory Pension Scheme.

Applications with a curriculum vitae together with the names and addresses of three referees, quoting reference FOP.53, should be sent to the Assistant Secretary (Establishment) by June 22. Further particulars are available on request.

978(A)

### OIL EXPLORATION IN SOUTH AFRICA

Soekor, the South African National Oil Exploration Company, requires experienced personnel to join its exploration teams based in Johannesburg.

#### GEOLOGISTS

Minimum qualification: B.Sc. (Honours) Geology. The ideal candidate would be an experienced Petroleum Geologist or Well-site Geologist / Mudlogger. Knowledge of sedimentology, structural geology, basin evaluation, geophysics and geological log interpretation would be a strong recommendation.

#### GEOPHYSICISTS

Geophysicists of high calibre, suitably qualified and experienced in the field of reflection seismic interpretation and with a substantial knowledge of structural and sedimentary geology, are required.

#### PALYNOLOGISTS

Minimum qualifications: M.Sc. The work involves analysis of samples, ranging from Tertiary to Upper Jurassic. Appropriate experience in microspores and/or dinoflagellates will be an advantage. The successful candidate will form part of a team of micropalaeontologists.

Competitive salaries—negotiable. Benefits include assistance with travelling and resettling costs, holiday bonus, home ownership through low interest loans, group pension, life assurance and a low cost medical aid scheme.

Write, giving full personal, academic and experience details to: The Head, Personnel, Soekor, P.O. Box 3087, Johannesburg, 2000 South Africa. 867(A)

# Pathologists

We wish to recruit additional Pathologists, male or female, into the Safety of Medicines Department to join a team of Pathologists concerned in the safety evaluation of new drugs. Either medical or veterinary graduates.

The present Section of Pathology encompasses a full range of pathological techniques including electron microscopy, histochemistry, autoradiography and tissue culture. While experience in this field is desirable, those without immediate experience will be offered suitable training and encouraged to progress to higher qualifications.

The Pharmaceuticals Division is very attractively situated in rural North Cheshire,

within easy reach of a wide range of housing and main road and rail routes. Conditions of service, career opportunities, and assistance given to married persons in moving home are designed to attract and retain staff of high calibre.

Please write giving details of qualifications and experience, or requesting an application form

to:- Mr. M. F. Losse,  
Personnel Officer,  
Imperial Chemical  
Industries Ltd.,  
Pharmaceuticals Division,  
Mersey, Alderley Park,  
Nr Macclesfield,  
Cheshire.

888(A)



Public Health Laboratory Service  
**Centre for Applied Microbiology and  
 Research, Porton Down,  
 Salisbury, Wiltshire**

**THE SPECIAL PATHOGENS REFERENCE LABORATORY** has the following vacancies:

## Chief Medical Laboratory Scientific Officer

Applications are invited for experienced virologists with expertise in tissue culture, serology, and animal work. Previous experience in handling viruses known to be pathogenic for man would be an advantage.

The successful candidate will be expected to contribute to the research and development programme and to the routine work of the laboratory. He/she will be responsible to the Director of the laboratory and will be engaged in the following aspects of work.

**(a) Reference work**

- (i) To assist with isolation and identification of viruses from acute samples submitted for diagnosis, particularly samples from suspected cases of Lassa/Marburg/Ebola fevers.

- (ii) To assist with serological investigations on convalescent sera.

**(b) Research and Epidemiology**

- (i) Research and Epidemiology of Haemorrhagic fever viruses, particularly Lassa, Marburg and Ebola. Other viruses to be investigated include Rift Valley Fever, Congo, Korean haemorrhage fever and certain arenaviruses.

- (ii) To produce high quality mono-specific immune sera for diagnostic procedures.

## Medical Laboratory Scientific Officer

Applications are invited for experienced virologists with particular expertise in tissue culture, immunofluorescent techniques, serology and experimental animal work. Previous experience in the handling of viruses known to be highly pathogenic for man would be an advantage.

The successful candidate will be expected to contribute to the research and development programme and to the routine work of the laboratory. He/she will be responsible to the Director of the laboratory and will be engaged in the following aspects of work.

1. To assist in routine diagnosis either through virus isolation and/or by serological methods of severe viral infections.
2. To assist in the development of new and improved techniques for rapid diagnosis of viral infections.
3. To produce diagnostic reagents for use in regional laboratories.
4. To assist in research activities as directed by the Director and Deputy Director of the laboratory.

**N.H.S. terms and conditions of service. Applicants must be State Registered.**

**Salary scales:** Chief M.L.S.O., £5,472 to £6,192; M.L.S.O., £3,261 to £4,680.

These posts may be discussed with Dr E. Brown, 0280-610391, ext. 326.

Applications, in writing, giving full details of experience, qualifications and the names and addresses of two referees to: Mrs M. Bushby, Personnel Officer, C.A.M.R.

979(A)

**PH  
LS**

**Public Health Laboratory  
Service Board.**

## university of wales university college of swansea

### Senior Research Assistant

Applications are invited from persons with or just completing a Ph.D. for the post of **Senior Research Assistant in the Department of Botany and Microbiology**. The successful applicant will work with Professor P. J. Syrett on the nitrogen assimilation of marine phytoplanktonic algae with financial support from N.E.R.C. Applicants should have experience and interests in biochemical cellular physiology.

The appointment, which will date from October 1, 1979 and will be for one year in the first instance with the possibility of renewal for a further two years, will be on a scale up to £4,776 per annum, plus U.S.S./U.S.D.P.S. benefits.

Further particulars and application forms (two copies) may be obtained from the Personnel Officer, University College of Swansea, Singleton Park, Swansea SA2 8PP, to whom they should be returned by Friday, June 8, 1979. 876(A)

## UNIVERSITY OF THE WEST INDIES—JAMAICA

Applications are invited for the post of  
**LECTURER/  
 ASSISTANT LECTURER**

in the

### DEPARTMENT OF ZOOLOGY

Whilst applications are invited in any field of Zoology, preference may be given to a well qualified applicant with interests in ecology.

**Salary scale (under review):** Lecturer, J\$8,913 to J\$13,917 per annum; Assistant Lecturer, J\$7,236 to J\$7,926 per annum (£1=J\$3.60). Family passages; F.S.S.U.; Study and Travel Grant; Unfurnished accommodation will be let by the University at a rental of 10% of salary, or a housing allowance of 20% of salary will be paid.

Detailed applications (three copies) with curriculum vitae and naming three referees should be sent as soon as possible to the Registrar, University of the West Indies, Mona, Kingston 7, Jamaica.

Applicants resident in the U.K. should also send one copy to the Inter-University Council, 90-91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 859(A)

## LIVERPOOL POLYTECHNIC

### DEPARTMENT OF BIOLOGY

### S.R.C. RESEARCH TECHNICIAN

**Salary Scale:**

£2,511 to £2,967 plus £312 per annum  
 To work for up to three years on the genetics and molecular biology of photosynthetic bacteria.

Applicants should have or expect to obtain a degree/H.N.C. in a biological subject including some experience of microbial genetics.

Further details may be obtained from Dr V. A. Saunders (Liverpool Polytechnic, Department of Biology, Byrom Street, Liverpool L3 3AF. Tel: 051-207 3581, Ext: 6) to whom applications, including a curriculum vitae and the names of referees, should be sent.

Closing date: 14 days from the date of this publication.

Please quote reference LP/291.

935(A)

## THE UNIVERSITY OF THE SOUTH PACIFIC

Applications are invited for the post of

### SENIOR LECTURER/ LECTURER IN PHYSICS (Post 79/40)

Applicants should have a doctorate (or equivalent research experience) in Physics or a closely related discipline as well as teaching experience. The appointee will be required to contribute to the teaching and development of undergraduate courses in pure and applied physics and in related interdisciplinary areas such as science. Current research interests in physics are environmental physics; renewable energy supplies; telecommunications and ionospheric physics and the appointee will be expected to pursue research in one of these areas. Preference will be given to applicants with interests in either environmental physics or renewable energy supplies but applicants in any of the above fields will be considered. There are opportunities for consultancy work in the University Region through the projects of the University's Institute of Natural Resources and Marine Resources. Applicants should be available by January 1980.

**Salary scales:** Senior Lecturer: F\$11,960 to F\$14,135 per annum; Lecturer: F\$8,175 to F\$11,563 per annum. (£1=F\$1.74.) The British Government may supplement salaries in range £1,842 to £2,796 per annum (sterling) for married appointees and £444 to £1,164 per annum (sterling) for single appointees (reviewed annually and normally free from tax and provide children's educational allowances and holiday visit passages. Family passages; gratuity 15% of basic salary; superannuation; partly furnished accommodation at maximum rental of 12% of salary (under review); appointment allowance; contract for 3 years and renewable by mutual agreement.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to Registrar, University of South Pacific, P.O. Box 1168, Suva, Fiji, no later than June 22, 1979.

Applicants resident in the U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 825(A)

## UNIVERSITY OF NATAL DEPARTMENT OF GEOLOGY DURBAN

Applications are invited from suitably qualified persons, regardless of race or national origin for appointment to the post of

### LECTURER

or

### SENIOR LECTURER

in

### ENGINEERING GEOLOGY

**Salary in the range:**

**Senior Lecturer:**

R11 400 to R15 600 per annum

**Lecturer:**

R8 100 to R13 200 per annum

In addition to teaching duties the person appointed will be expected to be active in, and to supervise, research. There will be considerable opportunities to reorganise and co-ordinate existing courses in engineering geology. An interest in hydrogeology may be an advantage.

The grade of appointment and commencing salary notch will depend on the qualifications and experience of the successful applicant. In addition, an annual vacation savings bonus is payable, subject to Treasury regulations.

Application forms, further particulars of the post and information on pension, medical aid, housing loan and subsidy schemes, long leave conditions and travelling expenses on first appointment are obtainable from the Registrar, University of Natal, King George V Avenue, Durban, 4001, with whom applications, on the prescribed form, must be lodged not later than July 30, 1979, quoting the reference Adv. D33/79.

W125(A)

# Careers for young people in Research and Development

Redland Technology is a member of Redland Limited, the multi-national group of Companies which is principally concerned with the manufacture of building and construction materials.

Due to planned expansion, the New Technology and Product Development Centre at Horsham in Sussex has a number of immediate vacancies, as well as vacancies for additional staff to be appointed later in the year.

We are looking for men or women to be appointed as Support staff to our Materials Technologists working in a number of different disciplines. These positions would suit either school leavers with good 'O' or 'A' levels in Physics or Chemistry, or candidates with some experience in industry and relevant qualifications.

These jobs are interesting and varied; main duties will involve carrying out experimental work, testing and measuring under the control of a Materials Technologist. It is company policy to train support staff with a view to promotion to Technologist when the appropriate academic qualifications have been attained and, as such, suitable candidates will be offered day release courses to study. In addition, opportunities will arise to participate in both external and in-house training courses.

The Centre is based in a country environment on the outskirts of Horsham where working conditions are good.

Salaries are realistic and will depend initially on age and qualifications. Other benefits include a subsidised canteen, generous sick pay scheme, four weeks holiday a year and a Company bus service from Horsham.

Please apply for an application form and further details of the vacancies either by writing to or telephoning:

R. A. G. Poulton (Ref 7/79), Commercial and Personnel Manager,  
Redland Technology Limited, Graylands, Horsham, Sussex. Tel: Horsham 2351.

## Redland Technology

911(A)

## Centre for Applied Microbiology & Research, Porton Down, Wilts.

### Pathogenic Microbes Research Laboratory

## Top Grade Microbiologist

An experienced microbiologist is required in the above laboratory, which is concerned with research into mechanisms of microbial pathogenicity. A large proportion of the laboratory's research effort is currently directed to background studies for the development of an improved vaccine against *Bordetella pertussis*. Other programmes involve the antigenic changes related to growth conditions of bacteria, and studies of the mixed bacterial populations present in the mouth and the gut.

The successful applicant will have extensive experience in the study of pathogenic microbes, and a knowledge of one of the following fields would be particularly advantageous; continuous culture, bacterial cell wall chemistry, adhesion of microbes to cell surfaces.

## Senior Grade Microbiologist

The person appointed will carry out studies in continuous culture of mixed bacterial populations derived from the mouth, gut and respiratory tract. A knowledge of the isolation and characterisation of organisms derived from these ecosystems would be an advantage. Applicants should possess a postgraduate qualification and the ability to conduct a research programme.

## Medical Laboratory Scientific Officer

A young graduate or well qualified technician with experience in microbiology and/or biochemistry is required to assist in the study of the growth of pathogenic bacteria in continuous culture.

## Medical Laboratory Scientific Officer

A research technician, having a degree or H.N.C., is required to join a group working on antimicrobial defence mechanisms. Applicants should have had training and experience in immunology, microbiology or microbial biochemistry, together with experience of immunological techniques, particularly those relevant to cellular studies.

Salary Scales: Top Grade Microbiologist £8,877 to £10,347.

Senior Grade Microbiologist £5,451 to £6,837.

Medical Laboratory Scientific Officer £3,261 to £4,680.

N.H.S. terms and conditions of service will apply. Further details of the posts can be obtained from Professor D. O. Ellwood or Dr A. Baskerville, telephone Idmiston 610391.

Applications including curriculum vitae and the names and addresses of the referees should be sent to Mrs M. Bushby, Personnel Officer, CAMR, Porton Down, Salisbury, Wilts. Closing date for applications June 14, 1979. 912(A)

**PHLS**

**Public Health Laboratory Service Board.**

## THE UNIVERSITY OF LEEDS DEPARTMENT OF METALLURGY

Applications are invited for a post of

### EXPERIMENTAL OFFICER

to work in

### ELECTRON MICROSCOPY

to take overall responsibility for the electron optical facilities in the Department of Metallurgy. These facilities comprise three transmission microscopes, a scanning microscope with microanalytical attachment, and a photo-emission electron microscope. The post involves supervision of the maintenance of the instruments, training of operators, and development of the techniques in their application to Materials Science. Applicants should have graduate or equivalent qualifications and should have had considerable practical experience in electron microscopy. It is hoped to make an appointment with effect from October 1, 1979.

Salary at an appropriate point on the IB scale for Other Related Staff (£3,775 to £6,355 per annum) (under review with effect from October 1, 1979), according to age, qualifications and experience.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 70/3/D. Closing date for applications: June 21, 1979. 893(A)

## UNIVERSITY COLLEGE CARDIFF

Applications are invited for the post of

### RESEARCH ASSISTANT

in Neurobiology in the DEPARTMENT OF ZOOLOGY. Applicants should hold or expect to obtain this year a first or upper second class honours degree and will work with Dr R. S. Pickard on a project, funded by an S.R.C. grant, concerned with the anatomy and electrophysiology of the honeybee brain. The successful applicant will be encouraged to register for a higher degree subject to normal University regulations. Salary: £3,689 per annum. Duties to commence September 1979.

Applications (2 copies), together with the names and addresses of two referees, should be forwarded to the Vice-Principal (Administration) and Registrar, University College, P.O. Box 78, Cardiff CF1 1XL, from whom further particulars are available. Closing date July 1, 1979. Reference 1797. 946(A)

## BIRKBECK COLLEGE (University of London)

Application invited for RESEARCH ASSISTANT (Chemistry) on S.R.C. —project involving the application of Extended X-ray Absorption Fine Structure Spectroscopy (EXAFS) to anti-cancer drugs, anti-inflammatory drugs and protein complexes, using the Synchrotron Radiation Facilities at Daresbury and Hamburg. Data analysis will be on an interactive computer graphics system at Birkbeck, linked directly with the IBM 370/165 computer at Daresbury.

Appointment for up to three years from October 1, 1979; starting salary £5,412 per annum (including London Weighting). May suit postdoctoral physicist or physical chemist with an interest in biomedical problems. Applications (curriculum vitae and the names of two referees) should be sent to Dr P. J. Sadler, Chemistry Department, Birkbeck College, Malet Street, London, WC1E 7HX (Tel: 01-580-6622, ext. 262 or 326), from whom further details are available. 941(A)

## UNIVERSITY OF EXETER DEPARTMENT OF BIOLOGICAL SCIENCES (N.E.R.C. Research Project) POSTDOCTORAL RESEARCH ASSISTANT and TECHNICIAN (Grade 3)

Applications are invited for the posts of PDRA and Technician to investigate the effects of soil animals on microbial populations and nutrient cycling processes under the direction of Dr J. M. Anderson.

The PDRA post will be in the salary range £4,232 to £4,776 p.a. Applicants should have experience in soil microbiology and/or biochemistry.

The technician (salary range £2,931 to £3,336 p.a.) will be responsible for chemical analysis and support of the above position.

Both appointments will be for one year in the first instance, with the possibility of renewal for a further two years, commencing October 1, 1979.

Applications, stating the names of two referees, to Dr J. M. Anderson, Department of Biological Sciences, Hatherly Laboratory, Prince of Wales Road, Exeter, from whom further particulars may be obtained. Closing date for receipt of applications June 22, 1979. 953(A)

## UNIVERSITY OF CAMBRIDGE

### DEPARTMENT OF CLINICAL BIOCHEMISTRY

Applications are invited for a

### UNIVERSITY LECTURESHIP (Non-clinical)

in the Department of Clinical Biochemistry at Addenbrooke's Hospital, Cambridge. It is hoped to appoint a person whose main interest is in immunological aspects of cell membrane structure, function and pathology.

The appointment will be for three years with the possibility of reappointment to the retiring age.

The pensionable scale of stipends for a University Lecturer, not ordinarily resident in College, is £5,850 a year rising by twelve annual increments to £9,000. There is no grade of Senior Lecturer.

Further information about the duties and conditions of the appointment may be obtained from Mr J. F. Howe, Clinical School Office, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QO, to whom applications (fifteen copies) together with the names of three referees, should be sent so as to reach him not later than June 25, 1979. 974(A)

## UNIVERSITY OF NEWCASTLE UPON TYNE DEPARTMENTS OF ORGANIC CHEMISTRY AND MICROBIOLOGY RESEARCH ASSOCIATE

Applications are invited for a Medical Research Council sponsored post involving a chemotaxonomic study of clinically-significant micro-aerophilic actinomycetes and related bacteria in collaboration with Dr M. Goodfellow and Dr D. E. Minnikin. Candidates should have completed a Ph.D. or have equivalent research experience and preferably have expertise in lipid analyses and chromatographic techniques. The appointment will be for three years at a commencing salary of £4,382.

Applications, including curriculum vitae and names of two referees should be sent by June 15, 1979 to Dr D. E. Minnikin, Department of Organic Chemistry, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU. 877(A)



# AHMADU BELLO UNIVERSITY ZARIA, NIGERIA

FACULTY OF MEDICINE  
Vacancies exist at various levels  
FROM PROFESSOR TO  
LECTURERS

in the following departments: Radiology, Chemical Pathology, Immunology (in Medicine), Anaesthesia, Anatomy, Medicine (Neurology, Endocrinology), Pathology (Morbid Anatomy), Paediatrics, Surgery, Physiology.

**SALARIES:** Professor, G.L.16, N11,568 to N12,720; Senior Lecturer, G.L.13, N8,064 to N9,024; Lecturer I, G.L.12, N7,404 to N8,052; Lecturer II, G.L.10, N5,760 to N6,732. (N1= approximately 0.97p.)

**CONDITIONS OF SERVICE:** Appointments are either pensionable (for Nigerians only) or on contract of two years in the first instance. Fringe benefits include part-furnished accommodation, or housing allowance in lieu of accommodation, and a 25 per cent Contract Addition is payable to all expatriates.

**METHOD OF APPLICATION:** Candidates should send 4 copies of their curriculum vitae to the Principal Assistant Secretary (Recruitment), D001/606 Nigerian Universities Office, 180 Tottenham Court Road, London W1P 9LE, as soon as possible, preferably in time to arrange interviews at the end of May. The curriculum vitae should give the following information: Post desired, full name, date and place of birth, nationality, permanent address, telephone number, if any, current postal address and telephone number, if applicable, marital status, number and ages of children, educational institutions attended with dates, full qualifications plus copies of certificates, previous employment with dates, present employment with dates and salary, list of publications, names and addresses of three distinguished scholars in relevant field as referees, two passport photographs of self. Candidates are advised to request their referees to forward references on them directly to the London Office.

853(A)

# ICI/UNIVERSITY OF LEICESTER JOINT LABORATORY IN MOLECULAR GENETICS

The above laboratory has vacancies for postdoctoral molecular biologists to work on the expression of eukaryotic genes in bacteria. Applicants with experience relevant to the cloning of copy DNAs, such as the purification and translation of messenger RNAs or appropriate biochemistry, will be favoured.

Appointments will be for two years in the first instance, renewable for up to five years, with salaries on the scale of £4,232 to £7,145 (under review).

Applicants should send a detailed curriculum vitae and names of two referees to Professor W. J. Brammar, Department of Biochemistry, University of Leicester, Leicester LE1 7RH, from whom further details can be obtained.

818(A)



# SENIOR EDITOR, SCIENCE

Macmillan Education is a rapidly expanding Company within the Macmillan Group. The Publisher for the U.K. Secondary School Division is looking for a Senior Editor to run the Secondary School Science Programme. The successful applicant will ideally have a science degree and commissioning experience in educational publishing. He/she will be enthusiastic and possess initiative and a sound business acumen.

The job will be based at our Basingstoke offices and offers a competitive salary and Company Car.

Please write, with full career details including present salary to:

Tony Feldman, Publisher, Macmillan Education Limited, 4 Little Essex Street, London WC2R 3LF. 958(A)

# THE QUEEN'S UNIVERSITY OF BELFAST RESEARCH ASSISTANT DEPARTMENT OF MICROBIOLOGY & IMMUNOBIOLOGY

Applicants for this post should preferably have a first or upper second class honours degree in microbiology or a related science. Experience in the immunology of viruses and the use of tracer methods or in the physiology of anaerobic bacteria would be advantageous as research in these two fields are main interests in the department. The post is tenable for one year but may be renewable annually for a further five years. Salary scale (under review) £3,384 to £4,884 per annum with contributory pension rights under U.S.S.

Curriculum vitae, giving the names and addresses of two referees, should be sent to the Personnel Officer, The Queen's University of Belfast, BT7 1NN, Northern Ireland. Closing date: June 15, 1979. Please quote reference 79/N. 898(A)

# UNIVERSITY COLLEGE CARDIFF

Applications are invited for the post of  
**RESEARCH ASSISTANT**

in the DEPARTMENT OF ZOOLOGY. The post is supported by the N.E.R.C., to work on a project entitled "Intra-specific Mechanisms of Population Regulation in the Pine Aphid, *Cinara pinea* (Mordv.), with particular reference to the Role of Flight Behaviour". Applicants should possess or expect to obtain a good Honours degree in Zoology or related discipline and will be encouraged or register for a higher degree. The post is tenable for three years from August 1, 1979. Salary range: £3,689 to £4,232 per annum.

Applications (2 copies), together with the names and addresses of two referees, should be forwarded to the Vice-Principal (Administration) and Registrar, University College, P.O. Box 78, Cardiff CF1 1XL, from whom further particulars are available. Closing date June 21, 1979. Reference 1792. 945(A)

# COMMONWEALTH AGRICULTURAL BUREAUX Vacancy for SCIENTIFIC INFORMATION OFFICER

at the

# COMMONWEALTH INSTITUTE OF HELMINTHOLOGY The White House

103 St Peter's Street

St Albans, Herts., England AL1 3EW

**Duties:** Scientific Information Officer required in the Commonwealth Institute of Helminthology at St Albans to work on *Protozoological Abstracts*.

**Qualifications:** Essential qualifications are a degree in biology, several years postgraduate experience working on parasitic protozoa, a knowledge of at least one foreign language, the ability to write clear and concise English and an interest in bibliographical work.

**Salary:** The salary in the scale £2,839 to £5,448 (under review) plus Outer London Weighting and a compensatory allowance to offset personal contribution to superannuation.

**Application forms** and full particulars from:

Executive Director,  
Commonwealth Agricultural  
Bureaux,  
Farnham House,  
Farnham Royal,  
Slough SL2 3BN.

**Closing date for applications:** June 30, 1979. 957(A)

# NEW ZEALAND Scientist HEAD OFFICE

# Department of Labour

# DUTIES

To advise the Factory and Dangerous Goods and Explosives Inspectorates on all matters relating to chemical and other occupational health hazards and safety in industry, especially the hazards of dusts, liquids and gases (toxicity, explosibility and flammability) and other dangerous goods. The appointee will head a team of scientists. He or she will be required to travel within New Zealand to investigate accidents, incidents and problems.

The appointee will be expected to maintain close liaison with other Government departments and organisations to ensure a co-ordinated and informed approach to problems. Such liaison will extend to overseas organisations when necessary. The appointee must be capable of keeping up to date with world trends in relevant subjects and to research specific matters in depth. The appointee will be required to assist in preparing discussion drafts of new and revised legislation and Codes of Practice, and must have the ability to explain technical matters to non-technical persons on an individual or group basis. Some lecturing to staff will be required.

# QUALIFICATIONS

The position would particularly suit a chemical engineer or industrial chemist with a degree in one of these disciplines. Applicants with qualifications in other appropriate disciplines will be considered. Practical industry experience is highly desirable.

# SALARY

The salary payable will be up to \$NZ18,377 per annum plus a General Wage Order Adjustment of \$NZ365.00 per annum.

Interviews will be conducted during the first week of July by an officer from the Department of Labour in New Zealand.

Successful applicants will receive assistance with fares and transfer of personal baggage to New Zealand.

Full details and application forms can be obtained by writing to:

The Chief Migration Officer,  
New Zealand High Commission,  
New Zealand House,  
Haymarket, London SW1Y 4TQ

Quote reference IMM 1/14/1, in your enquiry.

Closing date for applications is June 18, 1979.

920(A)

# UNIVERSITY OF LEICESTER DEPARTMENT OF ZOOLOGY RESEARCH DEMONSTRATORSHIP

Applications are invited for a Research Demonstratorship tenable for three years commencing October 1, 1979. Candidates should have a good honours degree in Zoology or Biological Sciences, and a definite interest in carrying out research in the fields of Fish Behaviour or Cell Biology. The successful applicant will be required to register for full-time postgraduate studies towards the degree of Ph.D. in either of the above fields, and will be required to assist with demonstrating and tutorial work in the department for a maximum of 10 hours per week during term time.

Salary within the range £3,504 to £3,689 to £3,961 with superannuation benefits.

Applications with the names of two academic referees should be sent as soon as possible to Professor H. C. Macgregor, Department of Zoology, University of Leicester, Leicester LE1 7RH. 897(A)

# ROTHAMSTED EXPERIMENTAL STATION Harpden, Herts. AL5 2JQ SCIENTIFIC OFFICER

required in the Nematology Department to join a group working on the taxonomy and pathotypes of agriculturally important cyst-nematodes and their relatives, using morphological observation by light and scanning electron microscopy supplemented by experimental studies of host-range, hybridisation and other aspects of biology. Interest in taxonomy an advantage.

**Qualification:** Honours degree or equivalent in Biology, Zoology or Agricultural Zoology, or an ordinary degree with relevant experience.

**Appointment** in grade of Scientific Officer, salary scale £2,839 to £4,414 (Pay Award pending). Point of entry depending on qualifications and experience. Non-contributory superannuation.

Apply in writing to the Secretary, giving names and addresses of two referees and quoting Ref. 398 by June 14, 1979. Further details on request. 964(A)

# CSIRO AUSTRALIA

## Postdoctoral Research Fellow

### Fuel Geoscience Unit Canberra Act

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees—2,400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

**Field:** PETROLEUM GEOBIOLOGY

**General:** The Fuel Geoscience Unit conducts research relevant to exploration for fossil fuels—coal, petroleum, natural gas and oil shale—and to their assessment and extraction. The Baas Becking Geobiological Laboratory in Canberra is supported jointly by CSIRO, the Bureau of Mineral Resources and the Australian Mineral Industries Research Association, and is well equipped for the microbiological studies envisaged. The project is expected to be of three years' duration (subject to continuation of support) and the successful applicant will work with the existing group at the Baas Becking Laboratory in Canberra.

**Duties:** Initiate and carry out a research programme, in coordination with existing projects, to develop surfactants and viscosifiers by microbial processes under conditions relevant to natural petroleum reservoirs. The aim of the work is to enhance the economically recoverable proportion of petroleum from certain Australian petroleum occurrences.

**Qualifications:** A PhD degree or equivalent qualification supported by evidence of research capacity. Experience in multidisciplinary projects would be an advantage.

**Salary:** Research Scientist/Senior Research Scientist \$A15,422—\$A22,405 pa.

**Tenure:** The position is available for 12 months initially with the possibility of extension(s).

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 604/162 should reach The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 23rd June, 1979.

Applications in U.S.A. and Canada should be sent to The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, N.W., Washington, D.C., 20036, U.S.A.

917(A)

## UNIVERSITY OF BIRMINGHAM

### DEPARTMENT OF GEOGRAPHY

### TWO RESEARCH ASSOCIATES

S.S.R.C. Funded Project on Moorland Change and Upland Management in U.K. National Parks

Applications are invited for two graduate research associates to work with Dr M. L. Parry on the airphoto-mapping and computer-assisted analysis of moorland change. The appointments require experience in the use of either aerial photographs or computers in geographical or allied research.

Appointment will be from October 1, 1979 for two years in the salary range £3,775 to £5,488 per annum, plus superannuation. Maximum starting salary will not exceed £4,333.

Further particulars are available from the Assistant Registrar, Science and Engineering, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, to whom applications (three copies) including full curriculum vitae and naming three referees, should be sent by Friday, June 8, 1979.

Please quote reference NH7.

924(A)

## UNIVERSITY OF SYDNEY

### LECTURESHIP IN MICROBIOLOGY

Candidates should have knowledge of basic microbiology. A higher degree or equivalent with teaching and research experience given preference. Administrative and industrial experience also taken into consideration.

Department's facilities are adequate for most fields of microbiology research, except animal virology. Present research programmes include aspects of cytophysiology, ecology, genetics, serology and biological nitrogen fixation.

Appointment to commence early 1980.

The position is expected to be filled by a probationary appointment of three years, capable of leading to tenure, but if all the University's requirements for tenure are deemed to be satisfactorily met, tenure may be granted at the time of appointment.

Salary Range: \$A15,786 to £20,737 per annum.

Applications including curriculum vitae, list of publications and names of three referees by June 15, 1979 to the Registrar, University of Sydney, NSW 2006, Australia, from whom further information available. Information also available from Association of Commonwealth Universities (Adpts.), 36 Gordon Square, London WC1H 0PF.

962(A)

## SHEFFIELD CITY POLYTECHNIC

### DEPARTMENT OF BIOLOGICAL SCIENCES

### RESEARCH ASSOCIATE IN BIOLOGICAL SCIENCES

Salary Scale:

£4,101 to £6,558 per annum

This is one of two posts of Research Associate created in the Polytechnic to provide leadership and direction of research in specific areas. The research activities of the Department are concentrated mainly in the areas of Microbial Biochemistry, Animal Physiology and Biochemistry.

Applicants must be well qualified and experienced in one of the above areas and have previous experience of research supervision. Recent industrial experience would be advantageous.

The appointment is for a fixed period of three years.

Requests for an application form, in writing only please, to the Recruitment Section of the Personnel Department, Sheffield City Polytechnic, (Dept. N), Halfords House, Fitzalan Square, Sheffield S1 2BB. Completed forms should be returned by June 8, 1979.

921(A)

## FACULTY OF MEDICINE

### DEPARTMENT OF MEDICINE

### AHMADU BELLO

### UNIVERSITY

### ZARIA

R/APP/108/23/Vol. II

Applications are invited for the post of

### SENIOR LECTURER

(in Clinical Immunology)

and

### LECTURER

(in Clinical Immunology)

For the post of SENIOR LECTURER candidates must be medically qualified, have 10 years' postgraduate experience and hold a recognised higher degree in medicine. Some previous experience in clinical immunology is required. Both medically qualified and science graduates will be considered for the post of LECTURER. Medically qualified graduates must hold a recognised higher degree in medicine but previous experience in immunology is not essential. Non-Medical qualified candidates must hold a higher degree and have had considerable experience in immunology. Successful candidates will be expected to help in the teaching of immunology to undergraduates and postgraduates and to assist in the running of a clinical immunology service for the Ahmadu Bello University group of hospitals. There are excellent facilities for research in a well-equipped immunology laboratory. The current research programme of the laboratory, which is supported by the United Kingdom Medical Research Council, is concerned with immunology aspects of some of the locally important infectious diseases.

**SALARIES:** Senior Lecturer, GL.13, N9,064 to N9,024; Lecturer I, GL.12, N7,404 to N8,052; Lecturer II, GL.10, N5,760 to N6,732. N.B.: N1 = approx. 79p.

The appointment may be either on fixed term normally of two years contract, renewable by mutual agreement or till the retiring age of sixty (60). Economy-Class air passage will be paid for appointee, wife and up to five (5) children on appointment, on home leave by overseas staff every second year and on termination. Pension Scheme with a grant for local travel in intervening years, are the fringe benefits, and partly furnished accommodation at rental not exceeding 7 per cent of salary will be provided. Detailed applications, three (3) copies including a curriculum vitae with photocopies of all certificates and naming three (3) referees should be forwarded by airmail, not later than June 29, 1979 to the Registrar, Ahmadu Bello University, Zaria, Nigeria. Applicants resident in U.K. should also send one copy to N.U.C. London Office, 180 Tottenham Court Road, London W1P 9LE. 910(A)

## UNIVERSITY COLLEGE OF SWAZILAND

Applications are invited for the post of LECTURER

in the

### DEPARTMENT OF ANIMAL PRODUCTION AND HEALTH

Candidates should possess a Master's degree (or higher) in any branch of Animal Production. Preference will be given to those who have specialised in Animal Nutrition. Candidates should have had teaching experience, preferably in a University or College in a developing country. The appointee will be required to teach the subject matter of his/her competence at both the degree and diploma levels; to prepare teaching materials and aids; within the limits of the available time, to carry out research and development activities; to carry out related work assigned by the Head of Department or the Dean of the Faculty or any other competent authority.

Salary scale: E5,940 to E7,860 per annum (£1 sterling = E1.75). The British Government may supplement salary Government may supplement salary in range £1,650 to £2,184 per annum (sterling) for married appointee and £408 to £960 per annum (sterling) for single appointee (reviewed annually and normally free from tax) and provide children's education allowances and holiday visit passages. Family passages; 2-4 year contracts; if appointment for limited period 25% gratuity in lieu of superannuation for first 2 years and 27½% for second 2 years. 10% inducement payable for those not obtaining supplementation from other sources; education allowance; reasonable rented accommodation; biennial leave.

Detailed applications (2 copies) with curriculum vitae and naming three referees to be sent direct to Registrar, University College of Swaziland, Private Bag, Kwaluseni, Swaziland, by July 4, 1979.

Applicants resident in the U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 860(A)

## IMPERIAL COLLEGE FIELD STATION

### Silwood Park, Ascot

### TECHNICIAN GRADE 5

Required to work on an MRC sponsored project.

"The Sexual Development of Malarial Parasites." Suitably qualified persons should have experience in one of the following areas:— Tissue Culture, Electron Microscopy or Biochemistry. The appointment is for approximately two and a half years initially.

Starting salary £3,474 to £4,056. Five weeks annual leave, plus extra days at Christmas and Easter.

Apply with curriculum vitae and the names of two referees to Dr R. E. Sinden, Imperial College Field Station, Ashurst Lodge, Ascot, Berks by May 31, 1979. 909(A)

## UNIVERSITY OF OXFORD

### DEPARTMENT OF AGRICULTURAL SCIENCE

### RESEARCH ASSISTANT

Applications are invited for the post of Research Assistant in Soil Science tenable for two years from October 1, 1979. The salary will be on the grade 1B scale (£3,689 to £6,108 per annum).

The successful applicant will be expected to initiate studies in solute transfer between soil aggregates and surrounding voids. A background in the physical sciences with a knowledge of soil science would be suitable.

Applications giving full details of qualifications and experience and the names and addresses of two referees should be sent to the Administrator, Department of Agricultural Science, Parks Road, Oxford OX1 3PF by June 30, 1979. 976(A)

# UNIVERSITY OF AUCKLAND NEW ZEALAND

Applications are invited for the following full-time teaching position. Conditions of Appointment and Method of Application are available from the Assistant Registrar (Academic Appointments) at Auckland University, or from the Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF. Applications, in accordance with 'Method of Application', will be accepted at any time up to **June 30, 1979**.

Commencing salaries in accordance with qualifications and experience will be established within the Lecturers scale which is at present NZ\$11,529 per annum rising by annual increments to \$14,250 per annum. In normal circumstances, a Lecturer whose services have proved satisfactory to the Council may expect in due course to be given the status of a Senior Lecturer, salary maximum NZ\$16,780 per annum. At present all salaries are supplemented by a General Wage Order of NZ\$365 per annum.

## ANTHROPOLOGY

### Lectureship in Physical Anthropology

It is hoped to recruit a human population biologist but palaeoanthropologists will also be considered. Applicants should be competent in teaching genetics, ecology and evolutionary theory. The ability to teach quantitative methods would be an advantage. The teaching of these topics is integrated into the Prehistory and Physical Anthropology Section of the Department of Anthropology. 959(A)

# CITY OF LONDON POLYTECHNIC DEPARTMENT OF BIOLOGICAL SCIENCES ELECTRONICS TECHNICIAN GRADE 5

The City of London Polytechnic require, as soon as possible, an Electronics Technician for the design and construction of electronic physiological and neurophysiological apparatus for teaching and research, together with day-to-day servicing of neurophysiological teaching laboratory, and supervision of the electronics workshop. Candidates should possess an H.N.C. in an appropriate field.

The vacancy exists in our Biological Sciences Department which is located at Calcutta House, Old Castle Street close to Aldgate and Aldgate East Underground stations.

Starting salary, which will depend on qualifications and experience, will be within the scale of £3,675 to £4,212 inclusive (under review).

Further details and an application form can be obtained from the Staff Records Officer, City of London Polytechnic, 117/119 Houndsditch, London EC3A 7BU. Please quote reference number 79/18. 969(A)

## POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a post supported by MRC to work with Dr J. Mowbray on adenine nucleotide sequestration in cardiac muscle (see Biochem. J. 176, 485 (1978)). The sequestered material has been located and the project is to establish its chemical nature and to characterise the enzymes involved. Appointment will be for two years at a starting salary (range 1A) of £4,261 plus £502 London Allowance. Enquiries and applications (including a curriculum vitae and names of two referees) should be sent to Dr J. Mowbray, Biochemistry Dept. University College London, Gower Street, London WC1E 6BT. 967(A)

# THE UNIVERSITY OF LEEDS DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a temporary post of

## RESEARCH FELLOW

in the above Department to work with Dr D. A. Harris on a project supported by the S.R.C. on enzymology of ATP synthesis by mitochondria and/or Chloroplasts. The work will involve studies of chemical modification of isolated and membrane proteins, and rapid reaction kinetics. Candidates should already possess, or expect to be awarded, a Ph.D. degree, within the next few months. Experience with, or interest in computerised systems would be an advantage. The appointment will be made for a fixed period of three years with effect from October 1, 1979 (or as soon as possible thereafter).

Starting salary in the range £4,382 to £4,882 on the 1A scale for Research and Analogous Staff (£3,883 to £6,555). The salary scale is under review with effect from October 1, 1978.

Informal enquiries about the post will be welcomed by Dr D. A. Harris (Telephone (0532) 36171, ext. 86).

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 83/22/D. Closing date for applications: June 15, 1979.

764(A)

# GLASSHOUSE CROPS RESEARCH INSTITUTE

requires a

## PLANT BIOCHEMIST

or

## PHYSIOLOGIST

in the

## PLANT PHYSIOLOGY DEPARTMENT

to initiate a research programme on mechanism(s) through which responses to light are controlled by phytochrome and other photomorphogenic pigments.

Appointment in Higher Scientific Officer/Senior Scientific Officer grade depending on qualifications and experience. Salary scales £4,101 to £5,448 (H.S.O.) or £5,154 to £6,898 (S.S.O.), under review. Applicants should have First or Upper Second class Honours degree in Botany or Plant Biochemistry with at least two years postgraduate experience. Non-contributory superannuation scheme. Further details and application forms on request. Applications giving full biographical details and names and addresses of two referees to Secretary, Glasshouse Crops Research Institute, Worthing Road, Rustington, Littlehampton, West Sussex BN16 3PU, by May 31, 1979. 899(A)

# UNIVERSITY OF BRISTOL

Applications are invited for the post of

## TECHNICIAN GRADE 5

within the

## DEPARTMENT OF SURGERY

The person appointed would be in technical charge of the department's Immunology Laboratory which is sited at the University Medical School. This laboratory is concerned with the investigation and treatment of cancer. Minimum qualifications: H.N.C. or equivalent.

Salary £3,474 to £4,056 per annum according to age, qualifications and experience (scale under review).

Applications in writing, with the names of two referees, to Mr F. E. Badrick, Chief Technician, Department of Surgery, Bristol Royal Infirmary, Bristol BS2 8HW. Closing date: May 31, 1979. 918(A)

# Medical University of South Carolina DEPARTMENT OF ANATOMY

Three positions are available from September 1, 1979.

## Assistant professor

with substantial teaching experience in gross anatomy and neuroanatomy. The individual should have several years postdoctoral experience and research interests should focus on retinal development and plasticity in the developing rodent visual system.

## Assistant professor

with broad teaching experience in neurobiology. Several years of postdoctoral experience are required and research interests should centre around response properties and intracellular injection of visual cortical neurons. This is a temporary position.

## Instructor

with teaching experience in gross anatomy and neuroanatomy. Some postdoctoral experience is required. Research interests should include development of the avian visual system and transplantation of the developing rodent visual system. This is a temporary position.

Closing date for applications: May 23, 1979. Send curriculum vitae to: Dr. R. D. Lund, c/o Office of the Dean, College of Medicine, Medical University of South Carolina, 171 Ashley Avenue, Charleston, South Carolina 29403. An Equal Opportunity/Affirmative Action employer.

W130(A)

# ST. CHARLES' HOSPITAL EXMOOR STREET, LONDON W10 MEDICAL LABORATORY SCIENTIFIC OFFICER/JUNIOR MEDICAL LABORATORY SCIENTIFIC OFFICER BIOCHEMISTRY DEPARTMENT

The Laboratory has a special responsibility for hormone radioimmunoassay but full training is available within the District. Applicants should be state registered although a junior medical laboratory scientific officer studying for H.N.C. (Clinical Chemistry) will be considered.

For further details telephone Mr Vermont, Senior Chief Medical Laboratory Scientific Officer, 01-969 2488, ext. 357.

Application form available from Personnel Department, tel. no. 01-969 2488, ext. 354. 856(A)

# RESEARCH ASSOCIATE Biochemistry of Genetic Recombination

Postdoctoral position at University of Maryland, Baltimore County, for *in vivo* and *in vitro* studies of DNA recombination and repair. Extensive interaction and collaboration with molecular biology groups at nearby National Institute of Health and Johns Hopkins University. Experience in molecular genetics or nucleic acid biochemistry highly desirable but not essential. To begin Summer 1979; earlier or later dates possible. Salary: \$14,000 to \$15,000, plus fringe benefits. Candidates with M.S. degree and demonstrated capacity for independent research will be considered. Send vitae and two (2) letters of recommendation to: Dr John Hays, Department of Chemistry, UMBC, Catonsville, Md., U.S.A., 21228. UMBC is an Affirmative Action/Equal Opportunity Employer. W129(A)

# UNIVERSITY OF MELBOURNE CHAIR OF AGRICULTURAL ENGINEERING

Applications are invited for appointment to the Chair of Agricultural Engineering which will become vacant in April 1980, on the retirement of Professor C. G. E. Downing who was the first occupant of this chair.

SALARY: expected to be \$A33,061 per annum.

Further information about the position and the duties involved, including details of application procedure, superannuation, travel and removal expenses, housing assistance and conditions of appointment, is available from the Registrar, or from the Association of Commonwealth Universities (Apts.), 36 Gordon Square, London WC1H 0PF.

Applications close on **September 30, 1979**. 968(A)

UNIVERSITY OF BASLE  
DEPARTMENT OF PRE- AND PROTOHISTORY

Applications are invited for the

## Professorship in Prehistory and Environmental Archaeology

VACANCY AFTER SEPTEMBER 1980

Duties include:

**Teaching:** Palaeolithic, Mesolithic and if possible Neolithic periods, in the context of the ecology of the Pleistocene and Holocene; technology and mining in pre- and protohistorical times; archaeozoology; excavations.

**Research:** In the subjects taught.

Collaboration with the protohistorical and Roman section.

Applications including curriculum vitae, list of publications and teaching experience should be submitted before end of July 1979 to the Dean of the Faculty of Science.

Address: Dekanat der  
Philosophisch-Naturwissenschaftlichen Fakultät  
der Universität Basel  
Klingelbergstrasse 70  
CH-4056 BASEL. W124(A)

INSTITUTE OF  
LARYNGOLOGY AND OTOLOGY  
Gray's Inn Road, London WC1X 8DA  
**RESEARCH ASSISTANT**

required to join a team investigating hearing processes. The appointment will be for one year during which time the successful applicant will undertake an accoustical study involving hearing impaired subjects. Applicants should have a good science degree and a working knowledge of electronic measurement and computer processing techniques.

Salary: £4,152 per annum inclusive.

The appointment is in association with the Nuffield Hearing and Speech Centre.

Write for application form and job description to:

**Dr D. T. Kemp**  
Department of Audiology  
Institute of Laryngology and Otology

977(A)

THE POLYTECHNIC  
HUDDERSFIELD  
DEPARTMENT OF LIFE SCIENCES  
**PRINCIPAL LECTURER, SENIOR LECTURER OR  
LECTURER II—HUMAN ECOLOGY**  
Ref: ACA/343

Preference will be given to candidates with teaching and/or industrial experience and a proven research record and possessing a first degree in a Biological subject and a higher degree in Agriculture or Biological resource management. Experience of developing countries particularly in tropical resources would be advantageous.

Staff are expected to undertake activities, including research, in addition to teaching duties.

Salary: Principal Lecturer £7,047 to £7,818 (Bar) to £8,844.  
Senior Lecturer £6,051 to £7,065 (Bar) to £7,572.  
Lecturer II £4,101 to £6,558.

Further details and application forms, which should be returned by June 8, 1979, from the Personnel Office, The Polytechnic, Queensgate, Huddersfield HD1 3DH (Telephone 0484 22288 Ext 2225). 907(A)

CHAIRMAN  
of the  
DEPARTMENT OF  
PHYSIOLOGY  
WAYNE STATE  
UNIVERSITY  
SCHOOL OF MEDICINE

Creative and energetic leader to assume the chair of this large and More than 20 faculty members, large and modern research and teaching facilities. Applicants diverse department July 1, 1980, should have an established and productive research record, experience in teaching and administration, to be able to assume responsibility for the medical, allied medical and graduate teaching programmes of the department. Sex, race, religion or national origin will not be factors in the selection process.

Interested persons should reply by July 1, 1979 to:

Dr Bernard H. Marks,  
Department of Pharmacology,  
Wayne State University,  
School of Medicine,  
Detroit, Michigan 48201.  
W122(A)

UNIVERSITY OF  
NEWCASTLE UPON TYNE  
DEPARTMENT OF  
ORGANIC CHEMISTRY  
**RESEARCH ASSOCIATE**

Applications are invited for a Science Research Council sponsored post involving the systematic analysis of mycobacterial mycolic acids and related long-chain compounds in collaboration with Dr D. E. Minnikin. Candidates should have completed a Ph.D. or have equivalent research experience, hold a first class degree in Chemistry and preferably have expertise in the analysis of long-chain fatty acids. The appointment will be for three years at a commencing salary of £4,382.

Applications, including curriculum vitae and names of two referees, should be sent by June 15, 1979 to Dr D. E. Minnikin, Department of Organic Chemistry, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU. 873(A)

**RESEARCH FELLOWS IN  
ASTRONOMY  
ROYAL OBSERVATORY  
EDINBURGH**

There are vacancies at the Royal Observatory Edinburgh for Research Fellows. The Observatory is responsible for three national astronomical facilities; the UK 1.2m Schmidt Telescope in Australia, the 3.8m Infrared Telescope in Hawaii and the COSMOS machine at Edinburgh. In Edinburgh there are several thousand plates from the UK Schmidt Telescope and photographic laboratories equipped to exploit this data bank. There are extensive facilities for developing and testing new instruments.

The Royal Observatory manages and operates the telescopes in Australia and Hawaii, and some of the Research Fellows are expected to spend time abroad as members of the operating teams. Others are based in Edinburgh, where there are excellent library, computing and workshop facilities. The Observatory in Edinburgh also houses the Astronomy Department of Edinburgh University.

Appointments are generally for a period of three years. A fixed stipend is paid, ranging from £3,695 to £8,466 per annum.

Further details and application forms from the Personnel Officer, Royal Observatory, Blackford Hill, Edinburgh EH9 3HJ. 922(A)

**NATURAL ENVIRONMENT  
RESEARCH COUNCIL  
INSTITUTE FOR MARINE  
ENVIRONMENTAL RESEARCH  
PLYMOUTH  
ELECTRONICS  
DEVELOPMENT  
ENGINEER/PHYSICIST**

To take charge of a small Electronics Group, design and develop new instrumentation for marine research, advise and assist staff of the Institute regarding their instrumentation requirements and maintain existing equipment.

Qualifications: A degree or HNC/HND plus experience in electronic engineering including analogue and digital circuit design; experience in microprocessor systems an advantage; ability to work at sea, occasionally, in research vessels and small boats an advantage.

Salary (under review): Scientific Officer to £2,839 to £4,415 or, for candidates with at least two years' postgraduate experience, Higher Scientific Officer to £4,101 to £5,448. Starting salary may be above the minimum.

Staff of the Council are not Civil Servants but pay and conditions are similar to those of the Civil Service, including a non-contributory pension scheme.

For further particulars and application form, please write, quoting Post No. 931, to The Director, Institute for Marine Environmental Research, Prospect Place, The Hoe, Plymouth PL1 3DH. 940(A)

UNIVERSITY OF  
NOTTINGHAM  
School of Agriculture  
SUTTON BONINGTON,  
LOUGHBOROUGH  
DEPARTMENT OF PHYSIOLOGY  
AND ENVIRONMENTAL STUDIES

Applications are invited for the following post which exists within the Department.

**TECHNICIAN GRADE 5—  
ELECTRONICS**

The Technician will be responsible for the design and construction of electronic equipment used in the Environmental Physics section of the Department; and for the routine maintenance and repair of standard equipment in the Department as a whole. Candidates should possess O.N.C., O.N.D., or other recognised equivalent qualifications and appropriate experience is essential.

Salary on scale £3,474 to £4,056 per annum.

Write to the Secretary at the School for an application form quoting Post Ref: 79/10. 929(A)

UNIVERSITY OF  
LIVERPOOL  
DEPARTMENT OF BIOCHEMISTRY  
**RESEARCH ASSISTANT/  
SENIOR  
RESEARCH ASSISTANT**  
Affinity Chromatography

Applications are invited from chemists or biochemists for the above post in a multidisciplinary group working on the principles and applications of affinity techniques. The salary will be within the range £3,689 to £4,776 per annum depending on age and experience. The post is tenable for one year from October 1, 1979. Informal enquiries may be made to Dr. P. D. G. Dean, Tel: 051-707-6022, Ext. 3166.

Applications, together with the names of two referees, should be received not later than June 7, 1979, to The Registrar, The University, P.O. Box 147, Liverpool, L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/600/N. 892(A)



**UNIVERSITY COLLEGE  
DUBLIN  
DEPARTMENT OF ZOOLOGY  
SESSION 1979/80**

A vacancy has arisen for a staff member in the Department of Zoology for the 1979/80 Session. The appointment will be made at the level of either Assistant Lecturer or College Lecturer. Applicants should have teaching, practical and research experience. Preference will be given to candidates with teaching and practical experience in The Invertebrate Phyla (1st Year); Insecta, Reptilia, Aves and Limnology (2nd Year); Animal Behaviour (Honours Level); and also with research experience in Limnology.

This appointment is for one year, from October 1, 1979 to September 30, 1980.

The current salary scales are:

Assistant Lecturer: £4,210 to £6,875 per annum

College Lecturer: £6,610 to £8,486 per annum

Entry point on the relevant scale will be in accordance with qualifications and experience. There is a non-contributory pension scheme.

Prior to application, further information (including application procedure) should be obtained from the Secretary and Bursar, University College, Belfield, Dublin 4. Telephone enquiries 693244, ext. 431.

The latest date for receipt of completed applications is Friday, June 8, 1979. W128(A)

**UNIVERSITY OF GLASGOW  
DEPARTMENT OF  
BIOCHEMISTRY  
POSTDOCTORAL FELLOW**

There is a vacancy for a Postdoctoral Fellow to work in a Research Group in this department in association with the Department of Medical Cardiology, investigating the fundamental questions about the structure of myocardial cells, mechanisms of myofibrillar assembly and degradation, metabolic regulation and the molecular details of drug interactions.

Salary will be on range 1A of the scales for Research and Analogous Staff (£3,883 to £6,555, under review).

Applications, including a curriculum vitae and the names and addresses of two referees, should be sent to Dr J. Dow, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ.

In reply please quote Ref. No. 4455M. 871(A)

**BEDFORD COLLEGE  
(University of London)  
ELECTRON MICROSCOPY  
TECHNICIAN**

Technician Grade 7

required from September 1, 1979, to take overall responsibility for the supervision of, and the provision of a technical service from, the College's Electron Microscope Unit. Applicants must be highly skilled in all techniques related to the electron microscope investigation of biological material and in the maintenance of electron microscopes and associated ancillary equipment.

Gross salary within the range £5,162 to £5,735 per annum. Annual season ticket loan scheme.

Application forms from Senior Assistant Secretary (Personnel), Bedford College, Regent's Park, London NW1 4NS. Tel: 01-486 4400, ext. 312. 864(A)

**UNIVERSITY COLLEGE  
CARDIFF**

Applications are invited for the post of **LECTURER** in the

**DEPARTMENT OF ZOOLOGY**

Salary range: £3,883 to £7,754 p.a. Appointment will be made towards the lower end of salary scale. Duties to commence October 1979.

Applications (2 copies), together with the names and addresses of two referees should be forwarded to the Vice-Principal (Administration) and Registrar, University College, P.O. Box 78, Cardiff CF1 1XL, from whom further particulars are available. Closing date June 7, 1979. Reference 1794. 948(A)

**UNIVERSITY OF  
EDINBURGH  
DEPARTMENT OF  
GENETICS  
LECTURER**

The Department of Genetics has a vacancy for a Lecturer. Applications are particularly invited from those with qualifications and research interests in quantitative and/or population genetics, but other applicants may also be considered. The appointment may be taken up on October 1, 1979 or at any time during the following academic year. Salary will be on the scale £4,232 to £8,452 per annum with placing according to qualifications and experience. Superannuation under U.S.S.

Further particulars may be obtained from Professor J. R. S. Fincham, Department of Genetics, West Mains Road, Edinburgh EH9 3JN, and applications, with the names of two referees, should be submitted to the Secretary to the University, Old College, South Bridge, Edinburgh EH8 9YL by June 30, 1979. Please quote reference 1054. 872(A)

**UNIVERSITY OF SURREY  
DEPARTMENT OF HUMAN  
BIOLOGY AND HEALTH  
TECHNICIAN GRADE 3**

We need a Technician to help with the Research and Teaching activities of a vigorous and expanding department. Applicants should have a good practical knowledge of techniques in animal or human physiology, especially modern methods in electronic recording.

Applicants should be suitably qualified and have had several years' laboratory experience.

The department is in a new building in attractive surroundings, adjacent to an historic town with excellent recreational facilities on the campus and in the surrounding area.

Further particulars and application form for the post may be obtained from the Staff Officer, University of Surrey, Guildford, Surrey, GU2 5XH, or telephone Guildford 71281, ext. 452. 934(A)

**NATIONAL INSTITUTE  
FOR RESEARCH IN  
DAIRYING**

The Biochemistry Department requires a biochemist to work on the metabolism of body reserves during growth, pregnancy and lactation in ruminants. The work involves a study of protein and lipid metabolism in vitro of muscle and other organs taken from animals in different physiological states.

Candidates should have a first or upper second class Honours degree in biochemistry or physiology.

Appointment will be as Scientific Officer (£2,839 to £4,415) or Higher Scientific Officer (£4,101 to £5,448) according to experience. At least two years' relevant postgraduate research or other approved experience is required for appointment as HSO.

Further details and application forms are obtainable from the Secretary, NIRD, Shinfield, Reading RG2 9AT. Please quote reference 79/18. 795(A)

**QUEEN MARY COLLEGE  
University of London  
APPLIED MATHEMATICS  
DEPARTMENT  
Applications are invited for  
appointment as  
POSTDOCTORAL  
RESEARCH ASSISTANT**

to work with Professor I. C. Percival on an S.R.C.-supported project: Hamiltonian Dynamics of Systems with a finite number of degrees of freedom. Appointment for 2 years from October 1.

Initial salary in range £4,835 to £5,412 per annum (including London Allowance).

Please apply by letter, giving age, qualifications, experience and names of 2 referees, to The Registrar, (N) Queen Mary College, Mile End Road, London E1 4NS. 862(A)

# MRC

**Medical Research Council**

**M.R.C. CLINICAL RESEARCH CENTRE  
(NORTHWICK PARK HOSPITAL)  
Watford Road, Harrow, Middx. HA1 3UJ**

**SCIENTIFIC OFFICER**

required to work on primate models of psychiatric disease, including particularly schizophrenia. Applicants must have experience of behavioural work with primates, and some knowledge of neuropharmacology. They should also have an interest in the possible role of neurotropic viruses in the causation of psychiatric and neurological disease.

Potential applicants are invited to visit informally the C.R.C. and to discuss their research ideas with Dr T. Crow (Tel. No. 01-864 5311 Ext. 2754).

This is a limited term appointment for five years. Salary within the range £5,631 to £8,256 including L.A.

Application form and further details from Mrs J. Tucker-Bull (Ext. 2685). Quoting Ref. 125/1/A38. Closing date June 16, 887(A)

**GUY'S HOSPITAL  
MEDICAL SCHOOL  
GRADUATE  
RESEARCH ASSISTANT**

required for three years to work with Dr. D. C. Watts in the Biochemistry and Chemistry Department on an investigation into the biochemistry of the subunit interactions of the phosphagen kinases, supported on a grant from the Science Research Council. Salary on scale £3,716 to £3,988 to £4,262 plus £502 London Allowance. Applicants, who will be expected to register for a Ph.D. degree, should have a good honours degree with some knowledge of enzyme chemistry and, preferably, an enthusiasm for marine biology.

Apply in writing, with full curriculum vitae, to Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, quoting Ref. B.C.1. 883(A)

**GEOCHRONOLOGIST**

A Geochronology Laboratory is to be established at the University of Regina in the Department of Geology. The successful applicant will be required to design, set-up and operate a solid source mass spectrometry laboratory specialising in uranium-lead and zircon dating methods. The position will be of particular interest to persons with a strong physics background. A knowledge of solid state electronics, minicomputers and data handling. The position, which does not require any teaching or administrative duties, will commence as soon as a suitable candidate is found. Salary will be commensurate with qualifications and experience. Applications, which must indicate availability and salary expected, should be forwarded, along with three letters of reference, to Dr G. R. Parslow, Department of Geology, University of Regina, Regina, Saskatchewan, S4S 0A2, Canada (PRIOR TO JUNE 30, 1979). W121(A)

**THE UNIVERSITY OF  
MANCHESTER  
ELECTRON MICROSCOPE  
TECHNICIAN  
DEPARTMENT OF MEDICAL  
BIOCHEMISTRY**

required to work with a small research team led by Professor Scott on structure and function of connective tissue. In addition to experience of electron microscopy, an interest in biochemistry is desirable, and candidates should be qualified to at least O.N.C. level.

Commencing salary up to £3,315 per annum.

Applications should be sent to Professor J. E. Scott, Department of Medical Biochemistry, Stopford Building, The University, Manchester M13 9PT. 944(A)

**UNIVERSITY OF HULL  
DEPARTMENT OF GEOLOGY  
POSTGRADUATE  
RESEARCH ASSISTANT**

Applications are invited from graduates, or from those expecting to graduate this year, for the position of research assistant on a three-year N.E.R.C.-funded project on diagenesis and patterns of cementation in sandstones. The project commences on September 1, 1979.

Salary will be on the scale £3,684 to £5,321 per annum.

The person appointed may register for a higher degree, if suitably qualified.

Applicants with relevant qualifications in the geological sciences should write to Dr B. Waugh, Department of Geology, University of Hull, Hull HU6 7RX, before June 8, 1979, and should include a full curriculum vitae and the names of two referees. 879(A)

**UNIVERSITY OF GLASGOW  
DEPARTMENT OF BIOCHEMISTRY  
POSTDOCTORAL FELLOW**

There is a vacancy for a Postdoctoral Fellow to work in a Research Group in this department in association with the Department of Medical Cardiology, investigating the fundamental questions about the structure of myocardial cells, mechanisms of myofibrillar assembly and degradation, metabolic regulation and the molecular details of drug interactions.

Salary will be on range 1A of the scales for Research and Analogous Staff (£3,883 to £6,555, under review).

Applications, including a curriculum vitae and the names and addresses of two referees, should be sent to Dr J. Dow, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ.

In reply please quote Ref. No. 4455. 943(A)

**MEMORIAL UNIVERSITY  
OF NEWFOUNDLAND  
Canada  
ASSISTANT PROFESSOR  
(Research)**

required to join a project on the molecular comparison of genes specifying isofunctional catabolic enzymes in plasmids.

A candidate with a Ph.D. is required, and someone with experience of work with DNA and restriction enzymes is preferred. Contractual appointment for two years, in the first instance, on a salary scale commencing at \$16,950 per annum.

Applications, including a curriculum vitae and the names of two referees, should be sent to Dr E. A. Barnsley, Department of Biochemistry, Science Building, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1B 3X9. 914(A)

## Smith Kline - RIT

a SmithKline company

For our Immunobiology Department we wish to engage at short notice

### A VIROLOGIST

with several years' experience in the field of virology (technique of tissue culture, virus culture and of virological diagnosis). He (she) will be engaged in research and development in the field of virus vaccines.

The applicant should speak English fluently and must at least have a fair knowledge of French.

He (she) is preferably between 30 and 35 years old.

Should you be interested in this job, send your application with curriculum vitae to our Personnel Department, 13 rue du Tilleul, 1320 Genval, Belgium. W131(A)

### MEDICAL, DENTAL AND VETERINARY PHYSIOLOGISTS

The Department of Physiology at Tufts University Schools of Medicine, Dental Medicine and Veterinary Medicine is seeking physiologists for tenure-track positions at the assistant and associate professor levels. Of particular interest are persons with strong research in cell physiology and teaching ability in the areas of organ, comparative and avian physiology. The department is committed to developing research programs relating to the interests of the three schools so that persons with the M.D., D.M.D., D.V.M. and/or Ph.D. degrees are encouraged to apply. Applicants should send a letter describing their research and teaching interests, a curriculum vitae with publication list and the names of three references to: Dr. Geoffrey W. G. Sharp, Department of Physiology, Tufts University Schools of Medicine, Dental Medicine and Veterinary Medicine, 136 Harrison Avenue, Boston, Massachusetts 02111.

Tufts University is an Equal Opportunity/Affirmative Action Employer. W123(A)

### UNIVERSITY OF BIRMINGHAM DEPARTMENT OF GEOGRAPHY RESEARCH FELLOW AND RESEARCH ASSOCIATES (2)

Leverhulme Trust Research Project on Viking Settlement, climate and environmental change around the North Atlantic.

Applications are invited for three research appointments in connection with the above 3-year project.

#### 1. RESEARCH FELLOW (ARCHAEOLOGIST)

Preference will be given to postdoctoral candidates with a working knowledge of Viking settlement, particularly in Iceland, and experience of archaeological and/or geographical fieldwork techniques. Ability to use published Icelandic data would be an advantage. Salary on the scale £4,232 to £7,145 per annum, plus superannuation. Maximum starting salary will be £4,776. Post tenable from July 1, 1979.

#### 2. RESEARCH ASSOCIATES

Candidates should have a good honours degree with preferred specialism in botany or entomology and a general interest in archaeology. Salary on the scale £3,775 to £5,488 per annum, plus superannuation. Maximum starting salary will be £4,333. Post tenable from October 1, 1979.

Further particulars are available from the Assistant Registrar, Science and Engineering, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, to whom applications (three copies) including full curriculum vitae and naming three referees, should be sent by Friday, June 8, 1979. Please quote reference NH6. 925(A)

### UNIVERSITY OF BATH SCHOOL OF MATERIALS SCIENCE LECTURER in MATERIALS SCIENCE

The School offers an honours degree in Materials Science and has extensive teaching commitments in Engineering Schools of the University. The lecturer is required to share in this teaching programme and the ideal candidate would be one who has experience in the field of selection and evaluation of engineering materials and is familiar with engineering design procedures. A strong professional or academic record would be essential and the successful candidate would be expected to contribute actively to the School's research activities.

Application forms and further particulars available from the Personnel Officer, University of Bath, Bath BA2 7AY, quoting reference 79/104/N. Closing date for applications: June 18, 1979. 885(A)

### UNIVERSITY OF THE WEST INDIES—JAMAICA Applications are invited for the post of LECTURER/ ASSISTANT LECTURER in the DEPARTMENT OF BOTANY

Preference will be given to applicants who possess a first degree with majors in Botany and hold postgraduate qualifications or have interest and teaching experience in Plant Ecology and Taxonomy of Seed Plants. The appointee will be required to take up duties as soon as possible.

Salary scale (under review): Lecturer, J\$8,913 to J\$13,917 per annum; Assistant Lecturer, J\$7,236 to J\$7,926 per annum (£1=J\$3.60). Family passages; F.S.S.U.; Study and Travel Grant; Unfurnished accommodation will be let by the University at a rental of 10% of salary or a housing allowance of 20% of salary is payable.

Detailed applications (three copies) with curriculum vitae and naming 3 referees should be sent direct as soon as possible to the Registrar, University of the West Indies, Mona, Kingston 7, Jamaica.

Applicants resident in the U.K. should also send one copy to the Inter-University Council, 90-91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 857(A)

TECHNICIAN Grade 5, with knowledge of Cytology required to assist in both the research and undergraduate teaching laboratories in the Department of Botany and Microbiology. It is essential for applicants to have a sound knowledge of Cytological Techniques. Salary according to experience in range £3,998 to £4,580 inc. of London Weighting. Application form and further details from Personnel Officer (Technical Staff CD13), University College London, Gower St., WC1E 6BT. 913(A)

### UNIVERSITY OF BASEL DEPARTMENT OF STRUCTURAL BIOLOGY POSTDOCTORAL POSITION

Development of new *ab initio* methods for the theoretical study of large molecules and their application to problems in Pharmacology. Experience with Quantum Chemical methods and programming techniques desirable. Send resume and names of two referees to Dr E. L. Mehler, Department of Structural Biology, Biocenter, The University of Basle, CH-4056 Basle, Switzerland. W127(A)

### UNIVERSITY OF PAPUA NEW GUINEA Applications are invited for the post of SENIOR TECHNICIAN (ELECTRONICS) in the DEPARTMENT OF PHYSICS

The appointee will be expected to perform the following duties: Take charge of a small, modern and well equipped electronics workshop; to provide on-the-job training to National Technicians; maintain and calibrate a wide variety of electronic instrumentation; design and develop a digital and analogue instrumentation for teaching and research. Applicants should hold a Higher National Certificate or equivalent qualification in Electronic Engineering and have several years appropriate experience. Appointment will be at the level of Senior Technical Officer Grade 2.

Salary: K10,165 per annum (£1 sterling=K1.49). Family passages; baggage allowance; gratuity; various generous allowances.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to Secretary, Box 4820, University P.O., Papua New Guinea, by June 29, 1979.

Applicants resident in the U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 861(A)

### UNIVERSITY OF OXFORD NUFFIELD INSTITUTE FOR MEDICAL RESEARCH

A post is available to work on some aspect of the physiology of the embryo, fetus or newborn. Preference may be given to an applicant with interest in embryology or neurophysiology. The appointment, tenable from October 1, 1979, is for three years initially, and may be extended for a further two years (maximum). Salary on University Research Staff Scale II (£6,080 to £7,754 per annum) or IIX (£6,317 to £8,257 per annum).

Application or enquiries, with curriculum vitae and names of three referees to:

Professor G. S. Dawes,  
Nuffield Institute for  
Medical Research,  
Headley Way,  
Headington,  
Oxford OX3 9DS. 902(A)

### UNIVERSITY OF NEWCASTLE UPON TYNE DEPARTMENT OF GENETICS POSTDOCTORAL RESEARCH ASSOCIATE

Do operons exist in eukaryotic organisms? Applications are invited for the above post in an S.R.C.-financed study of gene clusters and their control using the fungus *Aspergillus nidulans*. Starting date as soon as possible and not later than November 1, 1979. Tenable for three years. Biochemical background an advantage. Starting salary within Range 1A Research scale (4,232 to £4,776).

Applications with names and addresses of two referees to Dr H. N. Arst, Department of Genetics, Ridley Building, The University, Newcastle upon Tyne NE1 7RU. 952(A)

### UNIVERSITY OF ST. ANDREWS DEPARTMENT OF PSYCHOLOGY Applications are invited for TWO LECTURESHIPS

in the Department of Psychology, tenable from September/October 1979. For one post candidates should have advanced training and experience to qualify them for an appointment in any area of Human Psychology, and for other a background in Social Psychology, Sociology, or allied disciplines.

Salary at appropriate point on scale £4,232 to £8,452 (under review), starting salary probably not above £6,108, plus F.S.S.U./U.S.S.

Applications (two copies preferably in typescript) with the names of three referees should be lodged by June 30, 1979, with the Establishments Officer, The University, College Gate, St. Andrews, Fife, from whom further particulars may be obtained. 972(A)

### UNIVERSITY OF BRISTOL BONE BIOMECHANICS A RESEARCH ASSISTANT

is required to participate in a project financed by the M.R.C. to investigate the effect of mechanical function on bone remodelling, particularly in relation to senile and disuse osteoporosis. The experiments will involve instrumentation of bones in vivo.

This is a postdoctoral appointment but applicants with other suitable experience will be considered.

Salary in the range 1A £4,505 (£4,776 to £5,041) p.a. (under review).

Applications, including a curriculum vitae and the names of two referees, should be sent before June 11 to Dr L. E. Lanyon, Department of Anatomy, University of Bristol, Park Row, Bristol BS1 5LS, U.K. 947(A)

### UNIVERSITY OF NEWCASTLE UPON TYNE DEMONSTRATORSHIP IN ORGANIC CHEMISTRY

Applications are invited for the above post in the Department of Organic Chemistry, tenable from September 1, 1979, or by arrangement. Applicants should have, or expect to have, a Ph.D. in Organic Chemistry. The successful applicant will be expected to assist with both undergraduate and postgraduate teaching and to carry out research of his or her own choice in an area of Organic Chemistry.

The appointment is for three years with salary on the Grade 1B (bar) scale: £3,384 to £4,882 (under review) according to age, qualifications and experience. Membership of the appropriate University superannuation scheme will be required.

Two copies of applications, together with the names and addresses of three referees, should be sent to Professor Sir James Baddiley, FRS, Microbiological Chemistry Research Laboratory, The University, Newcastle upon Tyne NE1 7RU. 875(A)

### POSTDOCTORAL RESEARCH ON HORMONE RECEPTORS

Areas of interest include interaction of glucagon with membrane receptors and the role of lipids as well as the development of a glucagon antagonist. Candidate should have experience with biological membranes, endocrinology or lipid or protein chemistry.

Applications with full curriculum vitae and names of two references to:

Dr R. M. Epand  
Department of Biochemistry  
McMaster University  
1200 Main Street West  
Hamilton, Ontario  
L8S 4J9, Canada. W115(A)

**THE  
UNIVERSITY COLLEGE  
OF WALES  
ABERYSTWYTH**

**DEPARTMENT OF GEOLOGY**  
Applications are invited for the post of

**PART-TIME  
DEMONSTRATOR**

tenable for an initial period of two years from October 1, 1979. A good Honours Degree in Geology is required and the post offers the opportunity to pursue research, which may lead to a higher Degree. Salary on commencement £3,000 per annum (under review) rising to £3,250 after twelve months.

Application forms and further particulars are obtainable from The Registrar, The University College of Wales, Old College, King Street, Aberystwyth, SY23 2AX, to whom completed forms should be submitted no later than June 4, 1979. 908(A)

**UNIVERSITY OF  
CAMBRIDGE**

Department of Biochemistry  
Applications are invited for a

**POSTDOCTORAL RESEARCH  
ASSISTANT (Range 1A)**

to work with Professor D. H. Northcote, F.R.S. on a project funded for three years by the Science Research Council. Whole plants and plant tissue cultures will be used to investigate the key metabolic step in polysaccharide synthesis during the induced and *in vivo* differentiation of plant tissue. The date of commencement for this post is October 1, 1979.

Applicants should send a curriculum vitae and names of two referees to: Professor D. H. Northcote, F.R.S. Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB1 1QW. 956(A)

**KINGSTON POLYTECHNIC  
SCHOOL OF CHEMICAL AND  
PHYSICAL SCIENCES  
SENIOR TECHNICIAN**

To be responsible for a Life Science laboratory, which is used for undergraduate teaching, research and project work. Duties include microbiology, plant studies and tissue culture work plus experimental development and investigational work. An important post requiring someone with initiative and enthusiasm. T3/4 grade up to £4,953 inclusive.

**TECHNICIAN**

To be responsible for a postgraduate research laboratory. Interesting post with wide variety of duties. Applicants will be expected to have at least 'A' level chemistry and be able to assist research students and staff. Other duties include maintenance and servicing of laboratory equipment and other general duties. T2 grade up to £3,972 inclusive.

Details and application forms from Assistant Registrar (personnel), Kingston upon Thames KT1 2EE. Tel: 01-549 1366. 975(A)

**EDITORIAL RESEARCH ASSISTANT** required to work with Professor G. Burnstock in the Department of Anatomy. Experience is required in biomedical research together with ability to edit scientific material. The position would also involve helping to co-ordinate the research activities of a group of research workers including postgraduates and postdoctorals from overseas. Salary range: £5,074 to £6,368 per annum plus £502 London Allowance. Applications, together with names of two referees and full curriculum vitae to Assistant Secretary (Personnel), University College London, Gower St., London WC1E 6BT. 906(A)

**UNIVERSITY OF  
SIERRA LEONE  
NJALA UNIVERSITY  
COLLEGE**

Applications are invited for two **LECTURERS** in the Department of Environmental Studies and Geography in the Faculty of Education.

**LECTURER IN  
CLIMATOLOGY**

Candidates should have at least a second degree with specialisation in Climatology. The appointee will teach undergraduate courses in Climatology, agro-climatology, and participate in research on local environments. An interest in biogeography, tropical hydrology and moisture problems is an advantage.

**LECTURER IN REGIONAL  
SCIENCE AND  
QUANTITATIVE METHODS**

Candidates should have at least a second degree with bias for spatial analysis. The appointee should be able to teach basic concepts of quantitative methods to second and third year students and act as supervisor for final year students preparing dissertations.

Candidates with an option for project evaluation, planning, locational analysis and rural development in third World Environments are preferred. Salary scale: Lecturer: Le4,488 to Le6,897 per annum. (£1 Sterling = Le2.21). Family passages; University Superannuation or contract terms; annual leave; car loan negotiated. Part-furnished accommodation at reasonable rental; various allowances. Detailed applications (copies) with curriculum vitae and naming three referees, should be sent direct to the Secretary, University Sierra Leone, Private Mail Bag, Freetown, Sierra Leone by July 11, 1979. Applicants resident in the UK should also send one copy of their application to the Inter-University Council, 90-91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 966(A)

**UNIVERSITY COLLEGE OF  
SWAZILAND**

Applications are invited for the post of

**LECTURER**

in the Department of Agricultural Economics. Candidates should possess an M.Sc. in Agricultural Economics with a major in Farm Management. Experience in Farm Management and/or in lecturing in this subject would be an additional recommendation. The appointee will lecture in Farm Accounts, Farm Management and in other topics relative to Agricultural Economics, as required. Salary scales: Lecturer I: E5,940 to E7,860 per annum. (£1 sterling = E1.75). The British Government may supplement salary by E1,650 to E2,184 per annum (sterling) for married appointee or E408 to E960 per annum (sterling) for single appointee (reviewed annually and normally free from tax) and provide children education allowances and holiday visit passages. Family passages; 2 to 4 year contracts; if appointment for limited period 25 per cent gratuity in lieu of superannuation for first 2 years and 27½ per cent for second 2 years; 10 per cent inducement allowance payable for those not obtaining supplementation from other sources; education allowance; reasonable rented accommodation; biennial leave. Detailed applications (2 copies) with curriculum vitae and naming three referees to be sent direct to Registrar, University College of Swaziland, Private Bag, Kwaluseni, Swaziland by July 11, 1979. Applicants resident in the UK should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London, W1P 0DT. Further details may be obtained from either address. 965(A)

**UNIVERSITY OF  
STIRLING  
DEPARTMENT OF  
BIOCHEMISTRY**

**TECHNICIAN GRADE 4**

Applications are invited for the above post. Previous experience in a Biochemistry Laboratory or related science laboratory essential. The main duties of the successful applicant will be to prepare practical classes and also assist in research in the department. Minimum of O.N.C. or equivalent qualification essential. Salary scale £3,222 to £3,708.

Application forms are available from the Establishments Office, University of Stirling, Stirling FK9 4LA. Tel: Stirling 3171. Ext: 2314. Closing date: June 4, 1979. 938(A)

**Academic Department  
of Child Health**

**The Charles Hayward  
Research Building  
Queen Elizabeth**

**Hospital for Children  
Hackney Road, London E2 8PS  
A JUNIOR  
MEDICAL LABORATORY  
SCIENTIFIC OFFICER B**

is required to carry out, under supervision, a research project investigating lymphoid cells in the small intestinal mucosa. The research will involve the application of immunological and cytological techniques to intestinal biopsy samples from children with gastrointestinal problems. A knowledge of immunology is desirable but not essential. Preferred qualifications—a Degree or H.N.C. in Biological Science. Minimum qualifications: two Science G.C.E. 'A' levels. Salary (at age 21 or over) £3,243 per annum, including London Weighting.

Application forms and further information can be obtained from Dr J. A. Walker-Smith. 904(A)

**UNIVERSITY OF OXFORD  
RESEARCH ASSOCIATE  
IN NEUROPHYSIOLOGY**

Applications are invited for this post to work on the activity of single neurones in visual, limbic, and motor areas of the primate brain. The post is appropriate for a postdoctoral scientist with experience in neurophysiology.

The salary is on the R.S. 1A salary scale, £3,883 to £6,555 (under review). Applications in writing to Dr E. T. Rolls, Department of Experimental Psychology, South Parks Road, Oxford OX1 3UD, England. 903(A)

**UNIVERSITY OF  
MANCHESTER  
RESEARCH ASSISTANT  
in the  
DEPARTMENT OF  
CHILD HEALTH**

Graduate with biological qualifications required to help with investigations into growth and development and its control. Salary range £3,689 to £5,321 per annum (£3,775 to £5,488 per annum from October, 1979). Applications with full personal details to Professor J. Dobbing, The Medical School, Oxford Road, Manchester M13 9PT. 915(A)

**POSTDOCTORAL POSITION** available 2 to 3 years for soil microbiologist interested in characterising tropical Rhizobia. Collaborative project with IITA Nigeria. Objective to increase nitrogen fixation by grain legumes in tropical farming systems. Send curriculum vitae and three references to Dr Allan Eaglesham, Boyce Thompson Institute at Cornell University, Ithaca NY 14853 USA. W117(A)

**ASSOCIATESHIPS**

**HERIOT-WATT  
UNIVERSITY**

**Riccarton  
DEPARTMENT OF CHEMISTRY  
S.R.C. POSTDOCTORAL  
RESEARCH  
ASSOCIATESHIP**

Applications are invited for the above post to study stereochemical aspects of some biosynthetic processes involving amino-acids. Applicants should be experienced synthetic organic chemists, with a strong interest in biological chemistry. The post is available for two years from October 1, 1979, but a later starting date would also be possible. The starting salary would be expected to be on the scale £4,333 to £4,910 per annum (subject to further revision).

Applications, giving the names of two referees, should be sent to Dr R. H. Wightman, Department of Chemistry, Heriot-Watt University, Edinburgh EH14 4AS, from whom further details can be obtained. 954(O)

**CONFERENCES**

**CALL FOR ABSTRACTS  
BIOPHYSICAL DISCUSSIONS  
Second Discussion—May 1980  
PROTEINS AND  
NUCLEOPROTEINS:  
STRUCTURE, DYNAMICS  
AND ASSEMBLY**

The Biophysical Society will hold its 2nd Biophysical Discussion at Airlee House, Airlee, Virginia (near Washington, D.C.) on May 18-21, 1980. This Discussion will consider recent advances in understanding the principles of macromolecular structure and dynamics. Sessions will be devoted to: elucidation of structure by diffraction and spectroscopic methods; nature of forces stabilizing macromolecular structure; fluctuations in macromolecular structures; and mechanisms of folding and assembly. Experimental systems to be discussed include proteins, viruses, and organelles involving protein-nucleic acid interactions.

Prior to the meeting, all participants will receive a study book containing the full Discussion papers and poster abstracts. There will be no formal presentation of papers at the meeting, only a five-minute reminder followed by open discussion. A \$175 fee will cover registration, three days' room and board, the study book, and the final proceedings volume.

Papers submitted for the Organizing Committee's consideration are due as follows:

July 16, 1979—Preliminary abstracts (<300 words, to be reviewed and selected by August 1st)

December 1, 1979—Complete manuscripts (to be refereed and selected by mid-January)

The full edited proceedings of ca. 500 pp, will be published in hardback by Rockefeller University Press (\$20 prepublication, \$30 after October 1980). Identical text will be received by *Biophysical Journal* subscribers (1980 subscription, 12 issues, \$150). For these publications, remit to Order Service, Rockefeller University Press, P.O. Box 5108, New York, N.Y. 10249, USA.

For further information contact Valerie Parsegian, Executive Secretary, Biophysical Discussions, P.O. Box 30239, Bethesda, Maryland 20014, USA. Phone (202) 362-8184. W93(C)

## CONFERENCES—continued

## THREE IMPORTANT CONFERENCES

25 and 26 September 1979

### ADVANCES IN MICROBIOLOGICAL TECHNIQUES Foods, Pharmaceuticals and Cosmetics

In an intensive programme experts from academic and industrial establishments will consider the latest techniques for the identification and enumeration of micro-organisms. There will also be an important session on standards.

3 and 4 October 1979

### PHOTOGRAPHY IN PRACTICE — GETTING SUCCESSFUL RESULTS FROM PHOTOGRAPHIC AND VIDEO TECHNIQUES IN SCIENCE AND TECHNOLOGY

This conference is intended to help the scientist or technologist who is not a professional photographer to use photography as an effect tool and to obtain improved results for visual aids. There will also be review papers on visual psychology and holography.

22 and 23 October 1979

### INDUSTRIAL APPLICATIONS OF ELECTRON MICROSCOPY with particular reference to quality control

In a comprehensive programme acknowledged experts will discuss the practical applications of electron microscopy in a wide range of industrial situations. In addition to examples from specific industries there will be papers on the types of information obtainable with EM.

The venue for all three conferences will be the Sudbury Conference Centre, London EC1 which is near St. Paul's Cathedral.

for further details contact:

Beverley Humphrey,  
Scientific Symposia Ltd.,  
UTP House, 33-35 Bowling Green Lane,  
London EC1R 0DA, U.K. Telex: 299049 UTPRES G 955(C)

## ENDOCRINOLOGY '79

16-19 July 1979

Organised by the Endocrinology Unit at the R.P.M.S. and held at the Royal College of Physicians in London

### PROVISIONAL PROGRAMME

MONDAY, JULY 16: A. G. E. Pearce, Peptides of the brain and gut; P. C. Emson, Neurochemical studies on several brain peptides; S. E. Bloom, The endorphins; I. MacIntyre, Inter-relation effects in bone and role in cancer hypercalcaemia.

TUESDAY, JULY 17: G. D. Whedon, Metabolic and endocrine hormone studies in various mammal space flights; W. A. Peck, The cellular basis of osteoporosis; H. W. Boyer, Advances in molecular biology: implications for endocrinology; F. Gautier, Characterisation of porcine parathyroid hormone mRNA, cDNA, and cDNA-pBR322 hybrid plasmids; H. M. Kronenberg, Studies of cloned DNA coding for preproparathyroid hormone R. A. Hallowell, Studies of expression of human growth hormone by *E. coli* K12; H. M. Goodman, The structure and evolution of several mammalian polypeptide hormone genes.

WEDNESDAY, JULY 18: R. J. Vane, Prostacyclin; H. W. Kosterlitz, Enkephalins: receptors, biosynthesis and release; D. Hudson, An investigation of enkephalin-receptor interaction employing isosterically modified analogues; J. Alumets, Enkephalin in intrinsic nerves and endocrine cells of the gut and in two rectal carcinoids; A. Dell, Neuropeptides: high resolution purification procedures and their application to the study of new opiate peptides and opiate peptide precursors; B. M. Austen: Pituitary granules contain a protease specific for consecutive basic residues; T. M. Badger, The effects of chronic treatment with LHRH and a LHRH agonist (D-Trp<sup>6</sup>-Pro<sup>9</sup>-NH<sub>2</sub>-LRF) on the pituitary-gonadal regulation of LH and FSH secretion; P. Bernd, Serotonin binding protein—localisation in parafollicular cells of the sheep thyroid; L. Orci, Studies in peptide hormone secretion.

THURSDAY, JULY 19: L. J. Deftos, Calcitonin: current aspects of physiology and pathophysiology; C. Nagant de Deuxchaisnes, Studies in osteomalacia; A. W. Norman, Recent advances in Vitamin D metabolism; B. E. C. Nordin, Role of Vitamin D and its metabolites in malabsorption of calcium in osteoporosis.

A. Canigga, V. S. Fang, M. C. Sheppard, F. G. Hawkins-Carranza, P. J. Meunier, R. Ziegler, M. F. Holick, O. H. Sørensen, J. A. Kanis, G. Mazzuoli, J. C. Ghazarian, J. H. Cort, C. Milet.

Details: P. Lindsay, Endocrinology '79  
R.P.M.S., Du Cane Road  
LONDON W12 0HS. Tel: 01-743 2030, ext. 457

782(C)

## COURSES

### UNIVERSITY OF SOUTHAMPTON M.Sc./Diploma in Biological and Chemical Methods in Pest Control

This one-year course, which commences in October, is open to graduates in Biology, Chemistry, Biochemistry, Agriculture or any related subject. It provides instruction in modern techniques and methods used in pest management, and is particularly suitable for graduates in Biology or Chemistry who wish to become proficient in relevant aspects of both subjects.

Further information may be obtained from Dr J. W. S. Bradshaw, Department of Biology, Building 3, The University, Southampton SO9 5NH, please quote reference number 1902/R/N 933(D)

## STUDENTSHIPS

### UNIVERSITY OF LEICESTER

#### RESEARCH STUDENTSHIP

available in the GENETICS DEPARTMENT for work leading to a Ph.D. in one of the following areas: biochemical and developmental genetics of mouse or *Physarum*; population genetics of small mammals; cell division, surface biogenesis and DNA replication (including plasmids) in bacteria; molecular structure of human and rabbit haemoglobin genes.

Further details and applications as soon as possible to Dr I. B. Holland. Applicants should have or hope to have at least a 2(i) degree in a biological science. 939(F)

### UNIVERSITY OF LIVERPOOL

#### DEPARTMENT OF IMMUNOLOGY UNIVERSITY

#### POSTGRADUATE

#### RESEARCH STUDENTSHIP

Applications are invited from good honours graduates in a biological science who expect to graduate this year for a University Postgraduate Research Studentship to investigate immunological resistance to tumour growth induced by pregnancy in experimental animals.

The studentship is tenable for three years, subject to satisfactory progress, commencing in October 1979 and the successful candidate will be registered for the degree of Ph.D.

Applications, together with the names of two referees, should be received as soon as possible, by The Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote Ref: RV/613/N. 950(F)

### UNIVERSITY OF LIVERPOOL DEPARTMENT OF BOTANY S.R.C. and

#### S.R.C./S.S.R.C. RESEARCH STUDENTSHIPS

Applications are invited for one S.R.C. Research Studentship and two S.R.C./S.S.R.C. Studentships from October 1979. The S.R.C. award may be used to study any area of Botany. The S.R.C./S.S.R.C. awards will normally involve a more applied project supervised jointly by the Botany and Industrial Studies Departments.

Applicants must possess or expect to gain a good honours degree in a biological science. Areas of research interest should be stated in the application.

Applications, including details of academic experience and the names of two referees, should be received not later than June 8, 1979, by The Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote Ref: RV/606/N. 889(F)

### UNIVERSITY OF NOTTINGHAM

#### FACULTY OF AGRICULTURAL SCIENCE S.R.C. (C.A.S.E.) RESEARCH STUDENTSHIP

Applications are invited for a Research Studentship to investigate the relationship between the structure and the texture and appearance of a new type of food gelling agent. The project is in conjunction with a leading food manufacturer and will be aimed at obtaining increased understanding of this novel material. The successful candidate will be required to adopt a multidisciplinary approach and work in close collaboration with the Company concerned.

The successful applicant will be expected to register for the degree of Ph.D. Candidates holding a degree in Chemistry, Physics, Food Science or Biochemistry should apply to Dr J. R. Mitchell, Department of Applied Biochemistry and Nutrition, University of Nottingham, School of Agriculture, Sutton Bonington, Loughborough, Leics. LE12 5RD, enclosing a brief curriculum vitae. 927(F)

### UNIVERSITY OF LONDON KING'S COLLEGE

#### ZOOLOGY DEPARTMENT

Applications are invited for

#### TWO S.R.C. STUDENTSHIPS

leading to a Ph.D. degree, from applicants who have, or expect to obtain this year, a first or upper second class degree in Zoology or Biology.

1. Q.U.O.T.A. Award, to undertake research in one of the following fields:—

(a) 'Relationship of low molecular weight RNAs to gene activity in *Amoeba*'. Supervisor: Dr S. E. Hawkins;

(b) 'Structural and physiological basis of autotomy in starfish'. Supervisor: Dr R. H. Emson;

(c) 'Instar development in 1-, 2- and 3-host ticks'. Supervisor: Professor Don R. Arthur.

2. C.A.S.E. Award, jointly with the Pest Infestation Control Laboratory of the Ministry of Agriculture, Fisheries and Food, for an investigation of the behavioural and physiological activity of odorous (pheromonal) compounds in the rat. Supervisor: Dr D. M. Stoddart.

Application forms and further particulars may be obtained from the Secretary, Zoology Department, King's College, Strand, London WC2R 2LS. 970(F)

### UNIVERSITY OF NOTTINGHAM DEPARTMENT OF THEORETICAL MECHANICS S.R.C.

#### RESEARCH STUDENTSHIPS

The Science Research Council is prepared this year to offer to suitable candidates a limited number of research studentships tenable in the above department. The main research interests in the department are in various aspects of the theoretical mechanics of solids and fluids. One of the awards is a C.A.S.E. award in collaboration with Rolls-Royce Ltd on 'Combustion modelling in reheat systems'. Candidates (including those expecting to graduate this year) should be applied mathematicians, or engineers or scientists with a strong mathematical background. The value and conditions of these awards will be as described in S.R.C. publications. Applications and requests for further details should be made to Professor A. J. M. Spencer as soon as possible. 880(F)



## STUDENTSHIPS—continued

UNIVERSITY OF  
LIVERPOOLDEPARTMENT OF BOTANY  
RESEARCH STUDENTSHIP

Applications are invited for a Research Studentship tenable from October 1979 to study Biochemical Properties of Protoplasts from *Aspergillus nidulans*.

Applicants must possess or expect to gain a good honours degree in a biological science or biochemistry, with experience in Mycology, Fungal Physiology or Microbiology.

Applications, including details of academic experience and the names of two referees, should be received not later than June 8, 1979, by The Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote Ref: RV/605/N. 890(F)

UNIVERSITY OF  
LIVERPOOLDEPARTMENT OF BOTANY  
N.E.R.C.

## RESEARCH STUDENTSHIPS

Applications are invited for two Research Studentships tenable from October 1979 to study (a) Plant Community Dynamics of Heathland Colonisation following Severe Disturbance and (b) Translocation and Nutrient Absorption by Rhizomorphs of Basidiomycetes.

Applicants must possess or expect to gain a good honours degree in a biological science, with experience in Ecology, Mycology or Plant Physiology.

Applications, including details of academic experience and the names of two referees, should be received not later than June 8, 1979, by The Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote Ref: RV/604/N. 891(F)

UNIVERSITY OF  
SOUTHAMPTON

## DEPARTMENT OF CHEMISTRY

Applications are invited for the following CASE Studentships:

- (1) Synthesis of Antibiotics (Professor R. C. Cookson with Glaxo-Allenburys Research Ltd).
- (2) Synthesis of Bridged Heterocyclic Annulenes and Diazepines (Dr J. M. Mellor with Roussel Labs Ltd).
- (3) Organometallic Complex-Catalysed Reactions of Isoprene (Professor R. Baker with Bush, Boake, Allen Ltd).
- (4) Biologically Active Compounds in Insect Tissue (Professor R. Baker and Dr J. W. S. Bradshaw with May & Baker Ltd).

The successful candidates will be expected to register for a higher degree.

Applications, including the names of two referees should be sent to the appropriate academic supervisor at the Chemistry Department, The University, Southampton SO9 5NH. 932(F)

S.R.C./C.A.S.E. RESEARCH  
STUDENTSHIPDEPARTMENT OF PLANT  
BIOLOGYUNIVERSITY OF  
NEWCASTLE UPON TYNE

Applications invited from persons with a first or upper second class honours degree, for a C.A.S.E. studentship in plant biochemistry. The project deals with photorespiration and is entitled "Metabolic consequences of excessive ammonia production in leaves". Dr A. J. Keys of Rothamsted Experimental Station will be co-supervisor.

Details available from Dr C. V. Givan, Department of Plant Biology, The University, Newcastle upon Tyne, NE1 7RU, to whom applications should be sent with names of two referees. 963(F)

UNIVERSITY OF SURREY  
DEPARTMENT OF  
BIOCHEMISTRY  
S.R.C. C.A.S.E.  
STUDENTSHIP

Applications are invited for a C.A.S.E. Studentship financed by S.R.C. in collaboration with Shell Toxicology Laboratory, Sittingbourne, Kent, tenable for three years and for work on

Molecular studies of the fidelity of DNA repair

Applicants must have or expect to obtain upper second or first class honours in Biochemistry or a related subject.

Applications with curriculum vitae and the names and addresses of two referees should be sent to Dr L. J. King before June 25, 1979.

Department of Biochemistry,  
University of Surrey,  
Guildford GU2 5XH.  
Tel: Guildford (0483) 71281, Ext. 499. 937(F)

UNIVERSITY OF  
ABERDEENDEPARTMENT OF  
PHYSIOLOGY

## S.R.C.

## RESEARCH STUDENTSHIP

Applications are invited from graduates, or those expecting to graduate this summer, for a S.R.C. Research Studentship. A variety of research topics are available in such areas as endocrinology, neurophysiology, hyperbaric physiology, electrophysiology of nerve and muscle, haemostatic mechanisms, respiratory physiology and muscle physiology.

Details of academic record, names of two referees and requests for further information should be sent to the Head of the Department of Physiology, University of Aberdeen, Marischal College, Broad Street, Aberdeen AB9 1AS. 901(F)

OXFORD POLYTECHNIC  
DEPARTMENT OF BIOLOGY

## S.R.C.

## RESEARCH STUDENTSHIP

Applications are invited for Science Research Council Studentships in the Department of Biology for three years from October 1, 1979.

The main research interests in the Department are in the areas of Biochemistry, Animal Physiology, Insect Physiology and Biochemical Ecology.

Applicants must hold, or expect to hold, a First or Upper Second Honours Degree in an appropriate discipline.

Applications with the names of two referees should be made as soon as possible to

David R. Mobbs,  
Head of Department of Biology,  
Oxford Polytechnic,  
Oxford. OX3 0BP.

from whom further details are available. 882(F)

N.E.R.C., Unit of Invertebrate  
Virology, OxfordN.E.R.C. RESEARCH  
STUDENTSHIP 1979

Applications are invited for a Natural Environment Research Council Studentship to work on the biological and biochemical characterization of small R.N.A. viruses of invertebrates. The Studentship will be tenable for up to three years from October 1979 and the successful applicant will be expected to read for a higher degree. Applicants should have or expect to obtain a first or upper class second degree in a biochemical or biological subject.

Applications should be made to Dr Norman F. Moore, N.E.R.C., Unit of Invertebrate Virology, 5, South Parks Road, Oxford OX1 3UB. 923(F)

LIVERPOOL POLYTECHNIC  
DEPARTMENT OF BIOLOGY

Applications are invited for the following *Research Studentships/Research Assistantships*, tenable for three years, in the Department of Biology. Applicants should hold or expect to obtain a first or upper second class honours degree in an appropriate biological subject. Successful applicants will be required to register for a higher degree with the possibility of this leading to a Ph.D.

1. Ministry of Agriculture, Fisheries and Food Research  
Studentships

## PLANT PATHOLOGY

Supervisor: Dr. T. M. Jeves

"Plant pathogenic *Phytophthora* species in the irrigation water of commercial nurseries."

## 2. Science Research Council C.A.S.E. Studentship

## MICROBIOLOGY-BIOCHEMISTRY

Supervisors: Dr. C. T. Calam and Dr. G. P. Sharples

"The physiology and fine structure of actinomycetes during antibiotic production."

This studentship is held in collaboration with I.C.I. Ltd., Pharmaceutical Division, Manchester.

## 3. Natural Environment Research Council Studentship

One studentship for studies on EITHER

## SOIL ECOLOGY

Supervisor: Dr. M. S. Luxton

"Ecology of the soil mites of coal shale waste heaps."

OR

## POLLUTION ECOLOGY

Supervisors: Dr. I. D. Hodgkinson and Dr. N. W. Lepp

"Studies on trace metal transfer from plants to invertebrate herbivore food chains."

The pollution ecology studentship will form a part of a team programme concerned with various aspects of trace metal pollution studies.

## 4. Science Research Council Studentship

One studentship for studies on one of the following areas:

## GENETICS

Supervisor: Dr. J. P. Margham

"Genetics of either flour beetles of the genus *Tribolium* or the yellow fever mosquito *Aedes aegypti*, with particular reference to insecticide resistance studies."

## DENDROCHRONOLOGY

Supervisor: Dr. M. K. Hughes

"The use of X-ray densitometric and other novel techniques in dating timbers from buildings and archaeological sites."

## AVIAN TAXONOMY

Supervisor: Dr. W. G. Hale

"Variations in egg shell structure in relation to the classification of the Charadriiformes."

## INVERTEBRATE DIGESTIVE PHYSIOLOGY

Supervisors: Dr. M. S. Luxton and Dr. R. Gibson

"The feeding biology and digestive physiology of *Arion hortensis*."

## 5. Natural Environment Research Council Assistantship

PALAEO LIMNOLOGY-FRESHWATER  
PALAEOECOLOGY

Supervisor: Dr. G. H. Evans

"The Late-Quaternary history of the diatom flora of two nutrient rich meres, Rostherne Mere (Cheshire) and Ellesmere (Shropshire), with particular reference to the history of eutrophication of these meres."

## 6. Local Authority Assistantship

## PLANT ECOLOGY

Supervisor: Dr. T. C. Marks

"The growth and reproduction of *Spartina townsendii* in the Ribble Estuary."

Letters of application, together with a full curriculum vitae and the names and addresses of three referees, should be sent to the appropriate named supervisor(s), from whom further details may be obtained, within three weeks of the appearance of this advertisement. The address for correspondence is:

Department of Biology,  
Liverpool Polytechnic,  
Byrom Street,  
Liverpool L3 3AF.

Informal telephone enquiries will be welcomed: Tel STD (051) 207 3581. Please Quote Reference LP/293. 936(F)

## STUDENTSHIPS—continued


**UNIVERSITY OF OXFORD**  
**M.R.C. STUDENTSHIP**
**NUFFIELD DEPARTMENT OF ANAESTHETICS**  
**RADCLIFFE INFIRMARY, OXFORD**

Applications are invited for a M.R.C./D.Phil. studentship in an active clinical research department, from September/October 1979. The studentship will be in applied respiratory physiology. It will deal with practical and theoretical problems of lung volume measurement and in-vivo blood-gas analysis in the animal laboratory, the Intensive Therapy Unit and the Operating Theatres.

Applicants should have, or expect to gain, a good honours degree in Physiology. Applications, enquiries and further details can be obtained from:

Dr C. E. W. Hahn or Dr A. M. S. Black,  
Nuffield Department of Anaesthetics,  
Radcliffe Infirmary,  
Oxford OX2 6HE.

Telephone: Oxford (0865) 49891  
Ext. 892 (Dr Hahn)  
Ext. 6069 (Dr Black).

973(F)

**UNIVERSITY OF READING**  
**DEPARTMENT OF FOOD SCIENCE**
**S.R.C. C.A.S.E. STUDENTSHIP**

(In association with Unilever Research Laboratory, Sharbrook)

Applications are invited from persons possessing, or expecting to obtain, an Honours Degree (I or II(i)) in a relevant science (e.g. Microbiology, Biochemistry, Chemistry, Food Science) and who wish to proceed to a higher degree by research.

The project is concerned with the mechanisms of the survival of *Salmonella* in foods.

Applications stating age and qualifications and naming two referees should be sent no later than June 8, 1979 to Dr W. F. Harrigan, Department of Food Science, University of Reading, London Road, RG1 5AQ. 886(F)

**UNIVERSITY OF READING**  
**DEPARTMENT OF ZOOLOGY**
**S.R.C. AND N.E.R.C. RESEARCH STUDENTSHIPS**

S.R.C. and N.E.R.C. supported Studentships are available in the following projects:

1. Effects of water on mechanics of soft tissues.
2. Epidemiology of parasites in wild mammals.
3. The physiology of abnormal development *in utero*.
4. Behaviour sequence analysis using observation and physiological monitoring.
5. Trace element metabolism of crayfish.

Applicants should write in the first instance to Professor K. Simkiss, Department of Zoology, University of Reading, Whiteknights, Reading RG6 2AJ giving a curriculum vitae and the names and addresses of two referees. Closing date will be June 6, 1979. 884(F)

**UNIVERSITY OF BIRMINGHAM**  
**DEPARTMENT OF BIOCHEMISTRY**  
**S.R.C./C.A.S.E. STUDENTSHIP**

This award (S.R.C. plus £400 per annum) will be tenable for three years. The student may register for a PhD. The research, on 'The Regulation of  $\alpha$ -Amylase in Pregerminating Wheat' will be in collaboration with the Flour Milling and Baking Research Association.

Applicants should have, or should expect to get, a First or Upper Second Class degree, and should contact Dr D. E. Briggs, Department of Biochemistry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT (Tel. 021-472-1301 ext. 2492).

951(F)

**UNIVERSITY OF OXFORD**  
**DEPARTMENT OF ASTROPHYSICS**  
**S.R.C. C.A.S.E. RESEARCH STUDENTSHIP**

Applications are invited from persons holding or expecting to receive a First or Upper Second Class Honours degree in physics or astronomy for a C.A.S.E. Studentship in conjunction with the National Physical Laboratory, Teddington, to work on the accurate determination of atomic transition probabilities, with applications in astrophysics, and on the accurate measurement of high temperatures by radiative techniques.

Applicants with the names and addresses of two referees and a curriculum vitae should be sent to Professor D. E. Blackwell, Department of Astrophysics, South Parks Road, Oxford OX1 3RQ. 841(F)

**CITY OF BIRMINGHAM**  
**POLYTECHNIC**  
**DEPARTMENT OF SCIENCE**  
**(BIOMEDICAL SCIENCE)**  
**M.R.C.**
**RESEARCH STUDENTSHIP**

Applications are invited for an M.R.C. Studentship commencing on October 1, 1979. The project is concerned with the investigation of malaria *Plasmodium berghei* to infected mouse blood for the presence of potential haemolytic factors. The work will involve a wide range of analytical techniques including isolation of parasites from host cells.

Applicants should have, or expect to obtain, a First or Upper Second Class Honours degree in Biochemistry, Biological Sciences or related subjects, and an interest in Parasitology. The value and conditions of the award will be those of M.R.C. studentships.

Applications giving a full curriculum vitae and names and addresses of two referees should be sent as soon as possible to Dr M. G. N. Angus, Department of Science, City of Birmingham Polytechnic, Franchise Street, Birmingham B42 2SU from whom further details may be obtained (Tel. 021-356-6911 Ext. 253). 865(F)

**UNIVERSITY OF LIVERPOOL**  
**DEPARTMENT OF PHYSIOLOGY**  
**C.A.S.E. STUDENTSHIP**

Applications are invited for an S.R.C. C.A.S.E. Studentship to work on the development and application of immunochemical methods in studies on the chemistry and metabolism of active peptides in brain and gut. The industrial sponsor will be Reckitt and Colman Ltd. Candidates should possess or expect to obtain a good honours degree in Biochemistry or a related subject and should have an interest in peptide synthesis.

Applications, together with the names of two referees, should be received as soon as possible, by The Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote Ref: RV/612/N. 949(F)

**UNIVERSITY OF DURHAM**  
**DEPARTMENT OF CHEMISTRY**  
**S.R.C. C.A.S.E. STUDENTSHIP**

Applications are invited for a C.A.S.E. studentship, in collaboration with A.E.R.E. Harwell, for the 'Study of Adsorbed Species Using Inelastic Neutron Scattering Spectroscopy'. The studentship is available from October 1979 for candidates interested in the vibrational spectroscopy and structure of small molecules adsorbed in zeolite frameworks and on metal powders. Experimental work will be carried out in Durham, A.E.R.E. Harwell and at the Institut Laue Langevin in Grenoble, France. It is expected that some of the experimental measurements will be made using the Harwell Linear Accelerator which is nearing completion.

This topic offers the opportunity to gain experience in a wide range of preparative, analytical and spectroscopic techniques, together with experience in using cryogenic, vacuum (medium and UHV) and complex, computer controlled experimental apparatus.

The research will be conducted under the supervision of Professor T. C. Waddington and Dr J. Howard. Standard S.R.C. rates plus £300 per annum for a suitably qualified candidate. Applicants should possess, or expect to obtain, a 1st or upper 2nd class honours degree in chemistry or chemical physics.

Further details can be obtained from Dr J. Howard, Chemistry Department, University of Durham, South Road, Durham DH1 3LE. 931(F)

**UNIVERSITY OF BATH**  
**SCHOOL OF BIOLOGICAL SCIENCES**  
**RESEARCH STUDENTSHIP**  
**IN PLANT PATHOLOGY/**  
**BIOCHEMISTRY**

Applications are invited from students having, or expecting, a good honours degree in biology or biochemistry (preferably including courses in plant pathology and plant physiology).

The project will investigate the role of plant cell walls in resistance to fungal pathogens, especially w.r.t. the interaction between host cell wall and microbial cell wall-degrading enzymes. The study will involve enzyme purification and characterisation, extraction of plant cell walls and lectins, growth of biotrophic fungi *in vitro* and electron microscopy.

The position is available from October 1979 and the applicant will register for a M.Sc./Ph.D.

Applications with curriculum vitae and names of two referees should be sent to Dr R. M. Cooper, Crop Protection Group, School of Biological Sciences, University of Bath, Bath BA2 7AY. 919(F)

**UNIVERSITY OF ABERDEEN**  
**S.R.C. STUDENTSHIP**

Applications are invited for a three-year research studentship awarded to this Department by the Science Research Council. Students interested in the fields of Vertebrate or Invertebrate Physiology or Evolutionary Ecology Geckos should write immediately to the Head of Department of Zoology, University of Aberdeen, Tillydrone Avenue, Aberdeen, AB9 2TN. 900(F)

**UNIVERSITY OF NOTTINGHAM**  
**School of Agriculture**  
**SUTTON BONINGTON**  
**LOUGHBOROUGH**  
**S.R.C. C.A.S.E. RESEARCH STUDENTSHIPS**

Applications are invited from persons holding or expecting to obtain a good honours degree in an appropriate field for the following awards.

Department of Agriculture and Horticulture (2 awards)

1. An investigation into the agronomical and physiological factors affecting the production of cereals for seed. Particular emphasis will be placed on optimising the production of numbers of grain suitable for seed. The collaborating body is Rothwell Plant Breeders. (Dr J. S. McLaren)
2. A study of environmental factors affecting cereal growth and development in relation to an accurate computer simulation model of field performance. The project will be primarily concerned with leaf growth, canopy development and water relations. The collaborating body is the Meteorological Office (Bracknell). (Dr J. S. McLaren).

Department of Applied Biochemistry and Nutrition.

The project is concerned with the synthesis and deposition of the proteins in wheat grains. The collaborating company is RHM Research Ltd. (Dr G. C. Blackwood)

Department of Physiology and Environmental Studies.

The project is concerned with physiological studies on seed germination in sugar beet. The collaborating company is a well known Sugar Beet Breeding Company. (Prof. W. J. Whittington and Dr D. Grierson).

Further details about these projects can be obtained from the members of staff mentioned but potential candidates are advised to submit as soon as possible a full curriculum vitae including previous education and current courses, the names and addresses of two referees and where possible a telephone number. 930(F)

## STUDENTSHIPS—continued

QUEEN ELIZABETH  
COLLEGE  
Kensington

(University of London)

N.E.R.C. STUDENTSHIP  
Species diversity in fluctuating  
and constant environments

Applications are invited for the above Studentship, tenable for a period of 3 years, under the direction of Dr M. J. Bazin, Department of Microbiology, Queen Elizabeth College, Campden Hill, London W8 7AH, to whom applicants should send a curriculum vitae together with the names and addresses of two referees.

863(F)

QUEEN ELIZABETH  
COLLEGE  
Kensington

(University of London)

DEPARTMENT OF PHYSIOLOGY

## S.R.C.

## RESEARCH STUDENTSHIP

Applications are invited from good Honours graduates in biological sciences (e.g., Physiology, Biochemistry) for a Ph.D. research studentship funded by the Science Research Council. The successful applicant will join Professor D. L. Yudilevich's research group which is currently investigating *in vivo* the transport of radioactively labelled organic molecules (amino acids, sugars, hormones and drugs) in the perfused placenta and salivary gland. The applicant would be encouraged to extend these studies to the blood-brain-barrier (pial capillaries) and will receive all the necessary assistance from the group.

The Physiology Department at Queen Elizabeth College is active in research and very well-equipped for the proposed project.

The studentship is tenable for three years starting October 1, 1979. Requests for further information and applications, including a full curriculum vitae and the names of two referees, should be made to Professor D. L. Yudilevich, Department of Physiology, Queen Elizabeth College, Campden Hill Road, London W8 7AH.

980(F)

## UNIVERSITY OF LEICESTER

DEPARTMENT OF PHYSICS

## RESEARCH STUDENTSHIP

An S.R.C. Studentship is available in the Condensed Matter (Experimental) Group for the study of Ionic Liquids. The project will involve the application of Neutron Diffraction techniques and use will be made of the neutron beam facilities at A.E.R.E., Harwell and the I.L.L., Grenoble. Data processing will be undertaken on the Cyber 73 at Leicester.

The studentship provides an opportunity to join an active research group that is currently engaged on a number of inter-related projects that are not only of great fundamental importance but are also relevant to the needs of modern technology.

All enquiries concerning this studentship should be made to Dr R. A. Howe, Department of Physics, University of Leicester LE1 7RH.

971(F)

## FELLOWSHIPS

## UNIVERSITY OF YORK

DEPARTMENT OF BIOLOGY

## A.R.C. POSTDOCTORAL

RESEARCH FELLOWSHIP  
IN CHLOROPLAST  
BIOCHEMISTRY

Applications are invited for a post-doctoral Research Fellowship to investigate the characteristics and control of chloroplast division in wheat leaves under the direction of Professor Rachel Leech. The project will involve the study of the role of the nuclear genome in the regulation of chloroplast division. Preference will be given to applicants with experience in both plant biochemistry and plant ultrastructure.

The appointment, which will be on Grade 1A of the national salary scales for research staff, will be for three years.

Informal enquiries and further particulars from Professor R. M. Leech, Department of Biology. Two copies of applications, giving a curriculum vitae and the names of two referees, should be sent by Friday, June 8, to the Registrar, University of York, Heslington, York, YO1 5DD. Please quote reference number 11/6105.

928(E)

## EMBO

## European Molecular Biology Organization

LONG TERM FELLOWSHIPS IN  
MOLECULAR BIOLOGY  
AUTUMN 1979 AWARDS

Next deadline: August 31, 1979

EMBO long term fellowships are initially awarded for one year. Applications for a renewal for a second year and subsequently in cases of exceptional scientific merit for a third year are considered.

To be eligible a candidate must hold a doctor's degree. Preference will be given to European and Israeli applicants wishing to work within Europe or Israel. EMBO long term fellowships are not, however, awarded for exchanges between laboratories within any one country. Applications for fellowships to be held outside Europe and Israel are considered but they have a lower priority, as do applications from non-European scientists wishing to work in Europe or Israel.

Successful applicants will be notified of their awards on October 29, 1979.

Further details and application forms may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, 69 Heidelberg 1, Postfach 1022.40, F.R. Germany.

W126(E)

CSIRO AUSTRALIA  
Postdoctoral Research Fellow  
Division of Materials Science  
Parkville Victoria

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees—2,400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

## Field: CATALYSIS

**General:** The Division is engaged in a broad area of Surface Science, Materials Research and Production Technology. It operates laboratories at Parkville, Fitzroy and Fishermen's Bend in Victoria and at Woodville in South Australia. The appointee will be located at the Catalysis and Surface Science Laboratory, University of Melbourne, Parkville, Victoria.

**Duties:** To participate in a research group studying catalytic processes for fuel synthesis, particularly the use of new zeolite catalysts.

**Qualifications:** A PhD degree in Chemistry or Chemical Engineering or equivalent qualifications.

**Salary:** Research Scientist \$A15,422—\$A18,904 pa or Senior Research Scientist \$A19,572—\$A22,405 pa.

**Tenure:** The Fellowship will be for a period of five years.

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 370/346 should reach The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 23rd June, 1979.

Applications in U.S.A. and Canada should be sent to The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, N.W., Washington, D.C., 20036, U.S.A.

916(A)

## An Taisce

## The Heritage Trust

## HERITAGE GARDENS FELLOWSHIP

An Taisce—the National Trust for Ireland intends to appoint a person to catalogue plant collections in Irish gardens of international importance and to undertake related research. The person, based at the National Botanic Gardens, Dublin, will be required to spend most of the time working in gardens throughout Ireland. The fellowship is for an initial one year period renewable annually up to three years. A University degree or diploma in botany or horticulture with experience in horticultural taxonomy and plant identification.

**Salary:** £3,500 to £5,000; assistance will be given with transport and accommodation in the field.

**Due to the present postal difficulties in the Irish Republic, intending applicants should telephone An Taisce (Dublin 681944) for further information and application details.**

W133(E)

## UNIVERSITY OF

SOUTHAMPTON

## Insect Chemistry

Two postdoctoral research fellowships are available for October 1, 1979, to work in a large group concerned with the Chemistry of Insects. Both of the positions will be concerned with Organic synthesis of biologically active compounds but other studies will also be associated with the isolation of compounds involved in insect communication. The post will involve some collaboration with Biologists but no previous experience of biology is required.

## Organic Chemistry

A fellowship is also available on a project concerned with the use of organometallic complexes in organic synthesis.

Salary within the range £4,333 to £4,910.

Applications, including brief curriculum vitae and the names of at least two referees, should be sent, as soon as possible, to Professor R. Baker, Chemistry Department, Southampton University, Southampton SO9 5NH. Ref. No. N. 878(E)

## UNIVERSITY OF

BIRMINGHAM

DEPARTMENT OF

MICROBIOLOGY

## POSTDOCTORAL

## RESEARCH FELLOWSHIP

Biochemist or chemical microbiologist needed to purify cell-wall components responsible for the resistance of gonococci to killing by human phagocytes. The project, which relates to a gonococcal vaccine, is supported by the Medical Research Council for three years from October 1, 1979.

Salary will be in the Research Fellowship 1A range £4,333 to £7,521, plus superannuation. Maximum starting salary will be £5,199.

For further details apply to Professor H. Smith, Department of Microbiology.

Applications (3 copies) including full curriculum vitae and naming three referees should be sent to the Assistant Registrar, Science and Engineering, P.O. Box 363, University of Birmingham B15 2TT, by Friday June 15, 1979.

Please quote reference NM5. 868(E)

## FELLOWSHIPS—continued

**UNIVERSITY OF  
SOUTHAMPTON  
SCHOOL OF BIOCHEMICAL  
AND PHYSIOLOGICAL SCIENCES**  
Applications are invited for a  
**POSTDOCTORAL  
RESEARCH FELLOWSHIP**

in the Nutritional Group of the School of Biochemical and Physiological Sciences to work in collaboration with Dr D. A. York on an investigation of membrane composition and function of obese mice.

Three years postgraduate experience in Biochemistry (preferably in some aspect of lipid or membrane research) is essential for this appointment, which will be for two years, commencing by September 1, 1979 or as soon as possible thereafter. Starting salary £4,776 per annum. USS benefits.

Applications in the form of curriculum vitae and the names of two referees should be sent as soon as possible to Mrs P. Vaughan-Smith, The University, Southampton, SO9 5NH, quoting reference 1072/R/N 874(E)

**UNIVERSITY OF WARWICK  
RESEARCH FELLOWSHIP  
IN CONTROL THEORY**

Applications are invited for the above post tenable for two years in the area of nonlinear control theory in the Control Theory Centre of the Department of Engineering. Applicants should have a Ph.D. in Control/Systems Theory or Mathematics with an interest in Control Theory. Salary on the Research Range 1A scale: £3,883 to £6,555 p.a. (under review). Application forms and further particulars from the Academic Registrar, University of Warwick to whom applications should be sent as soon as possible. Please quote Ref. No: 39/4A/79. 866(E)

**UNIVERSITY OF  
SOUTHAMPTON  
SCHOOL OF  
BIOCHEMICAL  
AND  
PHYSIOLOGICAL SCIENCES  
BIOCHEMISTRY DEPARTMENT  
POSTDOCTORAL  
RESEARCH FELLOW  
BIOORGANIC CHEMISTRY**

Applications are invited for a POSTDOCTORAL FELLOWSHIP in the field of Bioorganic Chemistry. The successful candidate will participate in studies in the Biosynthesis of Natural Compounds. Applicants should have at least three years postgraduate experience in synthetic organic chemistry and should also be conversant with some basic biochemical techniques. A knowledge of HPLC and the use of radioisotopes is also desirable. The work will entail the synthesis of labelled precursors and their incorporation into natural products in biological systems. The appointment is for one year in the first instance commencing immediately, with the possibility of an extension for a second year.

Starting salary £4,776 per annum. U.S.S. benefits.

Applications with date of birth, curriculum vitae and the names of three referees should be sent to Mrs P. Vaughan-Smith, The University, Southampton SO9 5NH, by June 15, 1979. Please quote reference 1074/R/N. 881(E)

**POSTDOCTORAL FELLOWSHIP:** candidates with experience in molecular biology, protein- or nucleic acid chemistry. Applications to: Dr Gernot Walter, Institut für Immunbiologie der Universität Freiburg, Stefan-Meier-Str. 8, D-7800 Freiburg, W-Germany. W132(E)

**UNIVERSITY OF WARWICK  
POSTDOCTORAL  
RESEARCH FELLOWSHIP  
in  
BIOLOGICAL SCIENCES**

Applications are invited for a position as Research Fellow in a small group studying the ecology of aquatic viruses. The project will involve studies of (a) environmental effects on bacteriophage multiplication, (b) phage infection of microbes attached to surfaces, (c) genetic interactions (phage-host and phage-phage) in aquatic habits. Ideally, applicants should have a Ph.D. in Microbiology. Previous experience with bacteriophages and/or an interest in mathematical modelling would be an advantage. The appointment is funded by the Natural Environmental Research Council for three years commencing in October 1979. The starting salary will be £3,883 on the Research Range 1A scale, £3,883 to £6,555 per annum (under review). Applications (two copies) either typewritten or in black ink, giving details of age, qualifications and experience and the names of two referees should be sent to the Academic Registrar, University of Warwick, Coventry CV4 7AL, quoting Ref. No: 41/A/79, as soon as possible. Informal enquiries to Dr S. B. Primrose, Department of Biological Sciences, by letter or telephone 0203 24011, ext. 6019. 869(E)

## GRANTS

**UNIVERSITY OF  
LEICESTER  
RESEARCH**

**leading to Higher Degrees in the  
DEPARTMENT OF CHEMISTRY**

Grants are available for research studies leading to the degree of Ph.D. Research is active in all branches of Chemistry, examples being: Organophosphorus Chemistry, Inorganic Fluorine Chemistry, all forms of Spectroscopy including E.s.r. Spectroscopy, Radiation Chemistry, and the Study of Solvation.

Applications are invited from persons holding, or hoping to be awarded during 1979, a degree with First or Second Class Honours (Upper Division) in Chemistry, or equivalent qualifications.

For further information and application forms please write to Professor M. C. R. Symons, Department of Chemistry, University of Leicester LE1 7RH. 896(H)

## ASSISTANTSHIPS

**UNIVERSITY OF GLASGOW  
DEPARTMENT OF  
MICROBIOLOGY  
RESEARCH ASSISTANT**

A postgraduate research assistantship, funded by the S.R.C., is available for work on the mechanism of action of the thymidine analogue bromodeoxyuridine during bacterial growth and differentiation. Applicants should possess, or expect to obtain, a degree in microbiology or related discipline and have an interest in microbial physiology and genetics.

The appointment is for two years, from September 1, 1979, with an initial salary of £3,689 per annum (under review) on scale 1B for research and analogous staff.

Applications, including a brief curriculum vitae and the names and addresses of two referees, should be sent to Dr J. E. Coote, Microbiology Department, University of Glasgow, Garscube Estate, Bearsden, Glasgow G61 1QH, as soon as possible.

In reply please quote Ref. No. 4457M. 870(P)

**UNIVERSITY OF GLASGOW  
RESEARCH  
ASSISTANTSHIP/  
LECTURESHIP IN PHYSICS**

The University of Glasgow wishes to expand its research on gravitational wave detection and offers a special appointment in the Department of Natural Philosophy for work in this field. For the first three years from October 1, 1979, the post will be supported by a research grant made by the S.R.C. to the University in support of the work of Professor R. W. P. Drever and his group and at the end of this period it will be taken over by the University as a tenured lectureship. The research programme includes development and use of gravitational wave detectors using laser interferometry and other techniques. Correlated observations made with gravitational wave detectors at Glasgow and at California Institute of Technology are envisaged. A suitable applicant might be an experimental physicist or electrical engineer with at least three years postdoctoral experience. Previous work on highly sensitive or high precision experiments in any field could be an advantage.

Initial appointment will be at Research Assistantship level within Range 1A of the Research and Analogous Staff Scales (£4,910 to £6,355), with placement according to qualifications and experience. Appropriate superannuation scheme will apply.

Further particulars may be obtained from the Secretary of the University Court (Room 18), University of Glasgow, Glasgow G12 8QQ, to whom applications (8 copies), giving the names and addresses of three referees, should be lodged on or before June 22, 1979.

In reply please quote Ref. No. 4459M. 942(P)

**UMIST  
RESEARCH ASSISTANT**

A Research Assistantship is available in the Department of Chemistry to work with Dr. C. A. McAuliffe on a project aimed at developing a facile reversible molecular hydrogen storage system based on simple transition metal complexes. This postdoctoral position would suit a synthetic transition metal chemist or a physical chemist interested in adsorption of gases on solid surfaces. An interest in spectroscopic techniques applicable to surface phenomena and in e.p.r. would be an advantage. The appointment will be initially for one year.

Salary is on the range £3,883 to £4,382 per annum (under review).

Application by letter, quoting reference CH/73/A1 including a curriculum vitae plus the names of two referees should be addressed to Dr. C. A. McAuliffe, Department of Chemistry, UMIST, P.O. Box 88, Manchester, M60 1QD (Phone 061-236 3311 Ext. 2098). 895(P)

**UNIVERSITY COLLEGE  
LONDON  
DEPARTMENT OF GEOLOGY  
ASSOCIATE  
RESEARCH ASSISTANTSHIP  
POSTDOCTORAL**

This post, funded by the N.E.R.C. over a 3-year period, is for an experimentalist to study the influence of cavities and cracks on mechanical properties of rocks under conditions simulating those in the Earth's crust and mantle. The research will utilise high pressure/high temperature apparatus already available at U.C.L. but the A.R.A. will be expected to develop techniques for measuring acoustic velocity in samples undergoing deformation. An interest in the theoretical interpretation of materials deformation and/or the study of microstructures by electron and optical microscopy will be welcome. The research is supported by good laboratory and workshop facilities, technical staff, and grant for equipment, and has important applications in crustal deformation and fracture, the movement of fluids in the crust, and in the interpretation of geophysical observations related to crustal structure and geodynamics.

Salary range £4,261 to £4,805 plus £502 London Allowance; U.S.S.

For further details write to Dr S. A. F. Murrell, Department of Geology, University College London, Gower St., London WC1E 6BT, to whom applications, including curriculum vitae and the names of two referees should be sent by July 20, 1979. 905(P)

**IMPERIAL COLLEGE OF  
SCIENCE AND  
TECHNOLOGY  
DEPARTMENT OF BIOCHEMISTRY  
POSTDOCTORAL  
RESEARCH ASSISTANTSHIP**

A postdoctoral Research Assistant is required for Freeze-Etch Investigations of Synapse and Cell Membrane Ultrastructure in Muscles affected by Muscular Dystrophy. Previous experience in Membrane Research and Electron Microscopy is desirable. The successful applicant will join an active, well-equipped research group, preferably starting on October 1, 1979.

The position, supported by the Wellcome Trust, is for an initial period of one year, with the possibility of extension for a second year. The starting salary (according to age and experience) will be between £4,762 and £5,835, including London Allowance and U.S.S. benefits.

Applications, including a curriculum vitae, the names of two referees and a full statement of research interests and experience, should be sent to Dr David Shotton before June 20, to the Department of Biochemistry, Imperial College, London SW7 2AZ, England, or subsequently to the Marine Biological Laboratory, Woods Hole, Mass. 02543, U.S.A. 926(P)

## SYMPOSIUM

**CATALYSIS AND CONTROL OF  
POLYMERISATION**

The 8th Biennial Manchester Polymer Symposium will be held at UMIST on Tuesday and Wednesday, April 1 and 2, 1980.

Aspects of the control of the structure of commodity polymers by the choice of Ziegler-Natta catalysts will be discussed on the first day. Invited Lecturers will include Dr D. Ballard (ICI Corporate Laboratory), Dr P. J. T. Tait (UMIST) and Professor P. Teyssie (Liège). Catalysts for speciality polymers will provide the topic for the second day when Invited Lecturers will include Professor G. Wegner (Freiburg) and Professor C. H. Bamford (Liverpool).

The programme will also provide time for a limited number of Contributed Papers. Offers of contributions should be sent to Dr A. S. Dunn at the UMIST Chemistry Department before July 16, 1979.

The symposium programme will be available in December 1979: requests for copies should be addressed to Barbara Halloran, UMIST, P.O. Box 88, Manchester M60 1QD. Tel. (061) 236 3311, Ext. 2753. 894(M)



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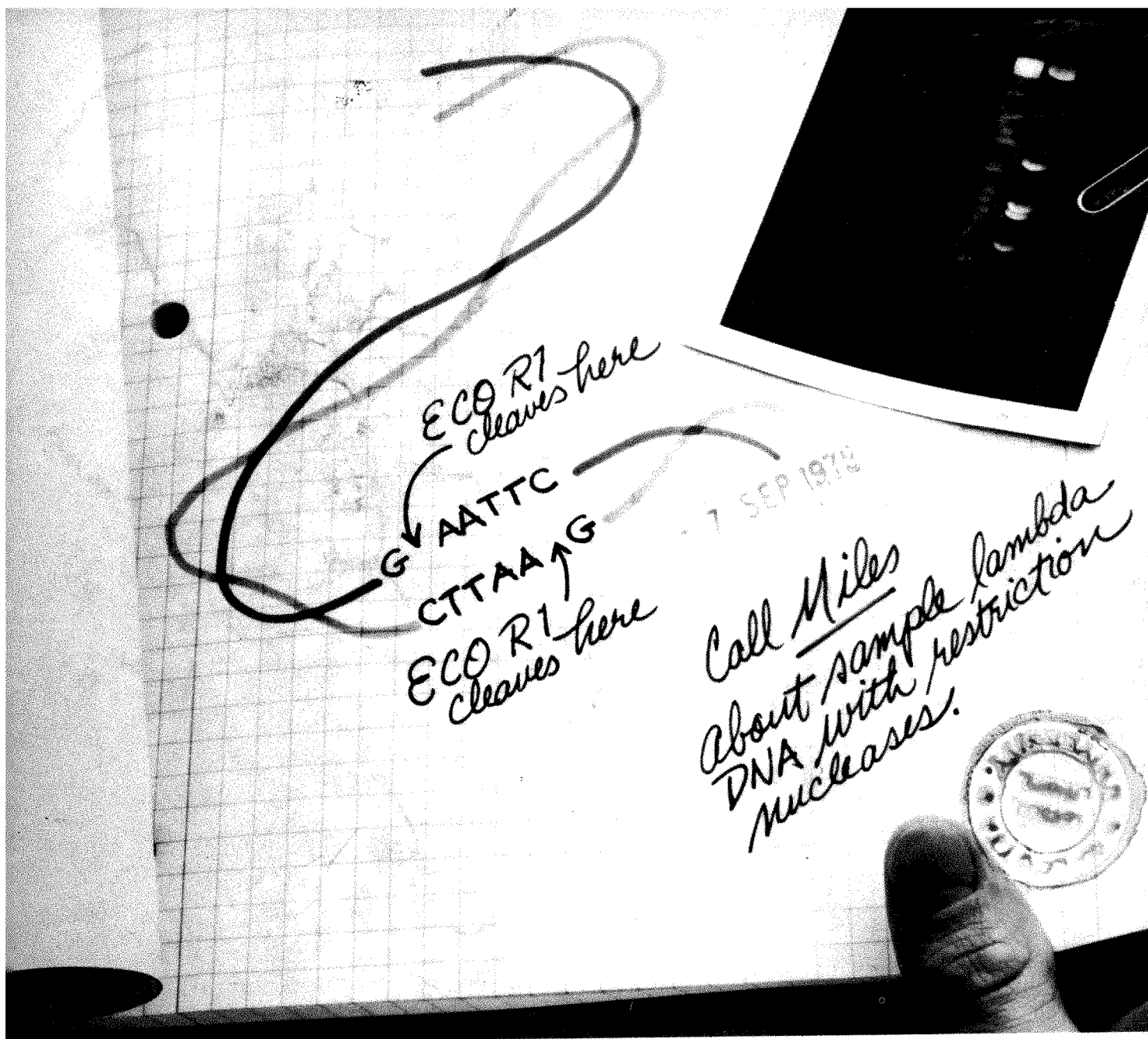
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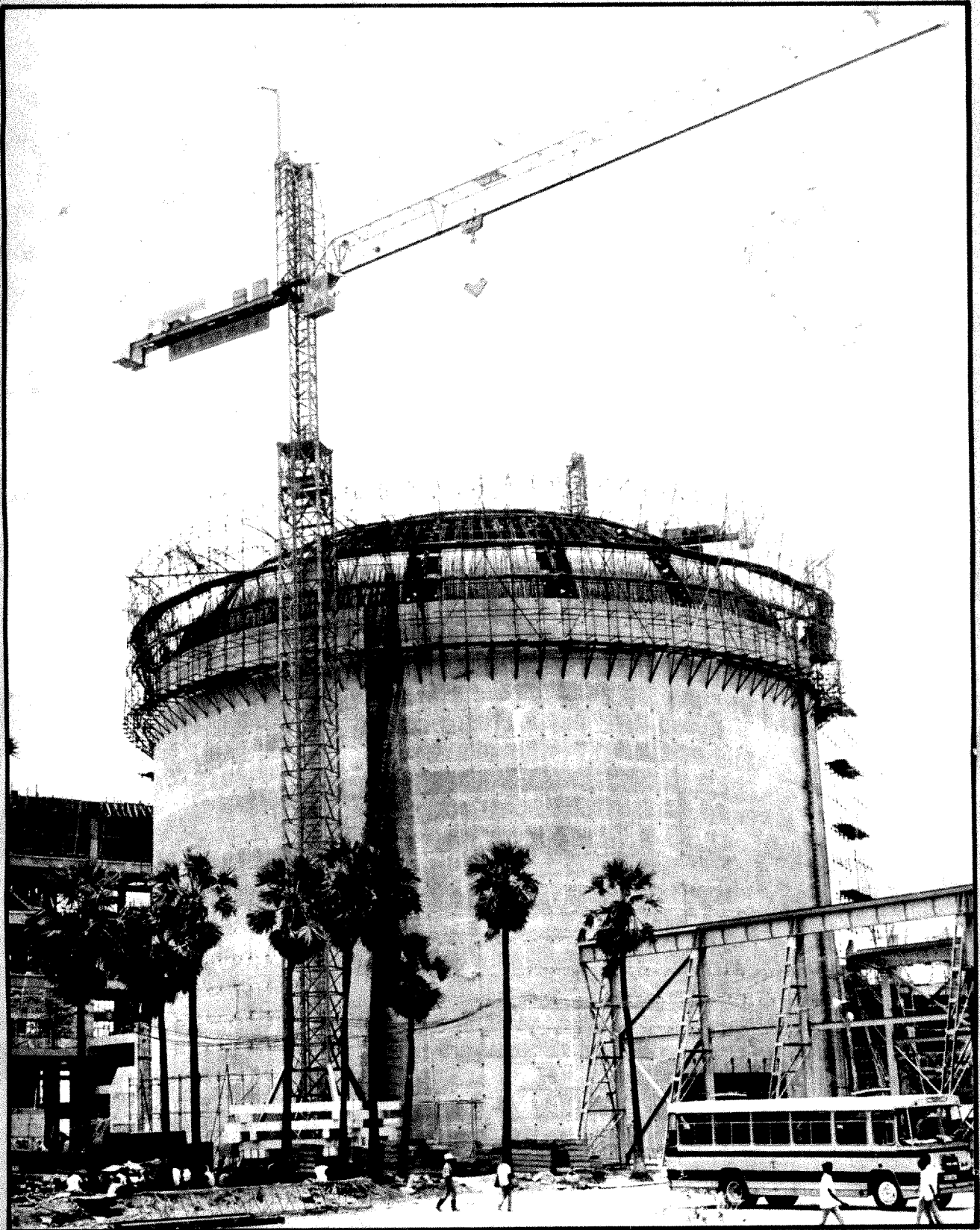


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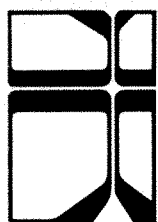
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# M



### : Solving the Mystery

**Professor John Imbrie and  
Katherine Palmer Imbrie**

*May 1979 £6.95 224pp 0 333 26767 2 illustrated*

Knowledge that the world once endured an ice age has been widespread for more than a century. Indeed, the concept is now so familiar that nearly every winter storm prompts dramatic headlines: Is a new ice age upon us?

This book tackles that burning question and attempts to unfold, for the interested general reader and those with a more academic interest, the entire saga of the Ice Age 'mystery' — why ice ages occurred, what they were like, and when the next one is due.

It is written by one of the principal scientists on the multi-million dollar CLIMAP project which set out to study changes in the climate of the earth over the past 700,000 years. Out of the group's analytical work came an exciting by-product: confirmation of the correctness of one of the several theories of what causes ice ages to occur. The theory that was confirmed, and is now explained along with many others in ICE AGES, is the Milankovitch, or astronomical theory which sees periodical glaciation as the result of three regular movements the earth makes as it travels around the sun: changing ellipticity of the earth's orbit, "wobble" of the earth's axis, and the changing tilt of the earth's axis.

"ICE AGES is fascinating in its scientific interest and also as a detective story. I recommend it to all." Dr. William F. Libby, Nobel Laureate.



For further information or copies of the above please contact: The Macmillan Press Ltd.,  
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M

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Construction of the Kalapakham  
nuclear power station near Madras,  
India. Anil Agarwal discusses on  
p 468 the problem of nuclear safety  
in the Third World, especially in the  
light of the recent accident at Three  
Mile Island.

Photo: India High Commission.

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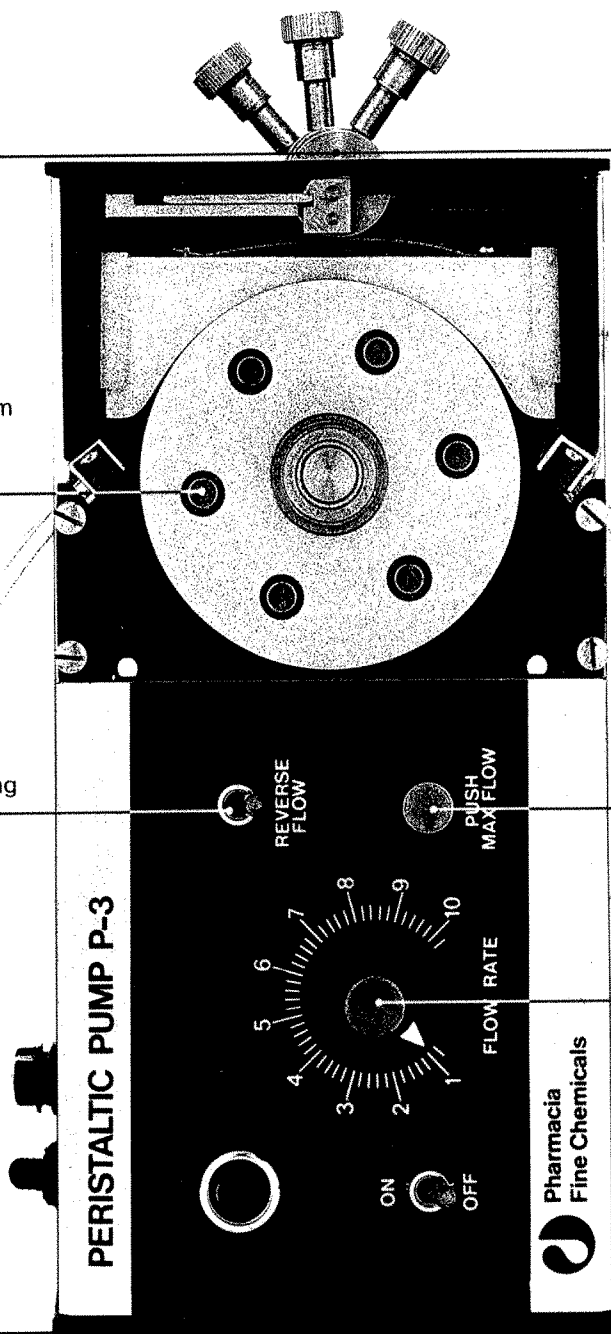
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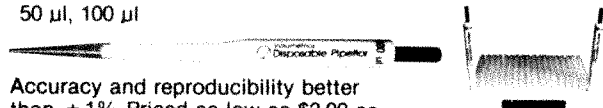
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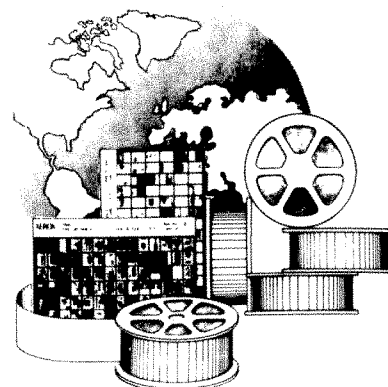
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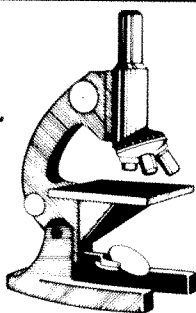
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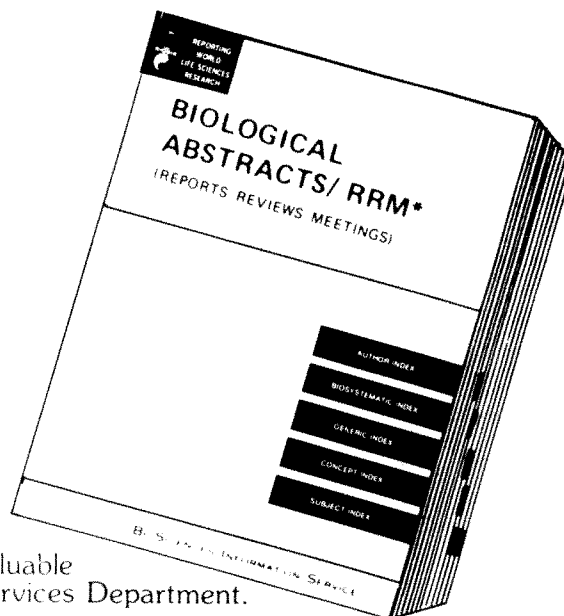
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**nature**

7 June 1979

## Recombinant DNA: now is the time for Congress to act

EVER since scientists first suggested that the uncertainties of recombinant DNA research made it advisable to agree on guidelines for containment levels, there has been no logical reason why research in private industry should be carried out under different conditions from university laboratories. After all, the essential concern has been over the potential health risks associated with this research, and such dangers do not differ from one context to another. Nor should one assume that industrial scientists are any more scrupulous than their academic colleagues.

There has thus always been a strong case for extending the guidelines established by the National Institutes of Health for federally-funded research workers in the US to cover the private sector as well (a member of the NIH's Recombinant DNA Advisory Committee recently called it "one of the most bizarre situations in the history of US science and technology" that this was not already the case). The essential question has not been whether this should be done, but how. And it has been the suggested answers to the 'how' questions, embroidering in various ways what might otherwise appear to be a relatively simple legislative concept, that have brought doom to a succession of congressional initiatives.

In the meantime, the process of developing, administering and revising the NIH guidelines has been proceeding relatively smoothly. Many scientists continue to believe that, even in their current form, the guidelines are too restrictive, and to baulk at the extra paper work involved. Others admit, however, that while some risk experiments have tended to confirm certain types of risks as being relatively small, others have indicated hitherto unanticipated potential hazards (such as the ability of DNA strands to induce tumours in laboratory animals). And that as long as these uncertainties remain, which itself is unpredictable, then some type of regulatory framework is necessary to cope with them.

For non-federally funded research, however, the picture remains confused. In the absence of new legislation, attempts have been made to fit the control of research using recombinant DNA techniques into existing legislative and institutional responsibilities. But the novelty of the hazards means that the fit has been an uncomfortable, and possibly untenable one. Thus Mr Califano, Secretary for Health, Education and Welfare, has said that he is reluctant to use powers granted to him under the Public Health Service Act, as some have been suggesting, as he is not sure whether the act is appropriate to the DNA case. In turn the Food and Drug Administration has decided that it probably lacks the legal power to require that all research leading to products submitted for licensing be carried out under the NIH guidelines as it had earlier been proposing to do.

The director of NIH has agreed that companies which express a willingness to comply voluntarily with the existing guidelines would be able to register with the NIH to have their research procedures approved. But the NIH is not, and has no desire to be, a regulatory agency.

(The Environmental Protection Agency and the Department of Labour's Occupational Safety and Health Administration have both kept relatively quiet on the issue, but in both cases there seems to be uncertainty over how to proceed with what remains a conjectural hazard.)

In the circumstances, there now seems a good chance that a new legislative initiative in the US Congress would be welcomed by many sides in the debate. To members of the Administration and federal institutions it would provide a clear delineation of roles and responsibilities in what will otherwise remain a murky situation. To private industry, although opposed in principle to government regulation, it should provide considerably more credibility than current promises to comply voluntarily with the NIH guidelines. And to environmentalists, public interest groups and labour unions, it would signal that private industry was not being given privileged status in the DNA debate (while also providing a channel, if minimal, for public participation in the type of debates that are constantly being urged on the future direction of new technologies with important social implications).

As with the previous legislative attempts, of course, the most controversial issues will inevitably be on the procedural, rather than the technical, aspects of complying with the guidelines. The fact that industry has already indicated that it is willing to comply voluntarily with the guidelines—and the extent to which industry spokesmen have gone to confirm that even at present, private research workers are following recommended physical and biological containment levels—shows that the inclusion of this aspect in new legislation should not provide too much of a problem. The sticky issues, however, will be over the appropriate forms of accountability and responsibility—over who should have access to the decision-making process, on what basis, and what should be done about data needed for the purposes of regulation but which a firm considers to contain information that would be valuable to a commercial competitor.

These are all political questions. At present they are being resolved by default by conventional means, with the decision-making agenda drawn up and controlled by those who stand to gain financially from a successful outcome to the research. It is up to those who, we hope, are now considering possible forms for legislation to decide whether this is the way they wish it to be, or whether to take bolder steps (such as involving trade union representatives in the decision-making process at a local level). It will take a political desire for change to carry it through, but a desire with which Mr Califano has already indicated his sympathy by expanding public interest representation of the RAC last December. RAC has, like Britain's Genetic Manipulation Advisory Group, been held up as a model pointing to the future direction of public participation in technological decision-making. Despite current limitations, we feel that this direction is the right one. And that regulation extending the NIH guidelines to industry should reflect this conviction. □

# Computers lose on the swings and the roundabouts

Tight university research budgets are contributing to a shortage of computer science graduates in the US. But concern is also growing about the social effects of automation.

**David Dickson reports**

NEXT Saturday, half a million members of the Communication Workers of America are holding a national "Job Pressures Day" to protest at the way in which automation is dehumanising working conditions. No one will stop work, the intention is to draw public attention to the issue through informational picketing and other activities under the general slogan "we are people not machines".

The action is claimed to be unprecedented for a major US union, most of which keep strictly to the conventional issues of wages and working hours. But it heralds a trend within the US labour movement which, fuelled by evidence that automation may finally be resulting in the unemployment previously predicted but unrealised, promises to grow substantially over the next few years.

Even without these new complaints about working conditions, the manpower implications of computers—a subject of hot debate in the late 1950s and 1960s, but one that subsided as economic prosperity cushioned any significant impact—are once again causing serious concern in policy-making circles.

On the one hand, support is declining for experimental computer science in US universities, and attracting good staff and students has become a problem. As a result, there are not enough qualified graduates for industry's needs. Many computer companies now face major difficulties in recruiting staff and have expressed their concern to the Office of Science and Technology Policy.

A report published last month by an advisory committee to the National Science Foundation says that as a result of funding difficulties, computer science research is now in a "critical" situation. University programmes are declining, just when the size and quality of research and training should be increasing to meet industry's needs. The report points out that, with obsolete equipment and inadequate finance, many of the best staff are now being recruited away from universities, whose research laboratories were originally responsible for such central innovations as time-sharing techniques, virtual memory systems and file protection mechanisms.

A particular concern, the committee says, is the fall in the number of computer science doctorates in the past

two years. "Because of its leverage in stimulating research, retaining faculty and capturing the interest of superior students, the most important requirement is the establishment and maintenance of outstanding university research facilities," it says. This could be achieved by injecting as little as \$15 million a year, based on five capital grants of \$2 million to separate institutions, plus running costs for each for the following five years. OSTP is now trying, with the support of industry, to persuade the Office of Management and Budget that this will be money well spent.

But while the management side of industry is complaining about a shortage of qualified recruits, labour is concerned about those who will lose their jobs as a result of automation. Until recently, the conventional wisdom was that unemployment was outweighed by the extra jobs created through increased prosperity. Now, with a stagnant economy plus the rapid incursion of automation into previously untouched areas such as office work, there are doubts about this equation.

A typical survey carried out by the Society of Manufacturing Engineers predicted recently that 20% of the labour directly involved in the final assembly of automobiles will be replaced by machines—such as robot

welders—by 1985, and 50% by 1995.

Similarly, a Bureau of Labour Statistics report on telecommunications technology concludes that "the anticipated strong demand for communications will probably modify the adverse effects on employment, but can no longer offset them. As a result, employment is expected to decrease slowly into the mid-1980s, significantly altering job skills and occupational composition."

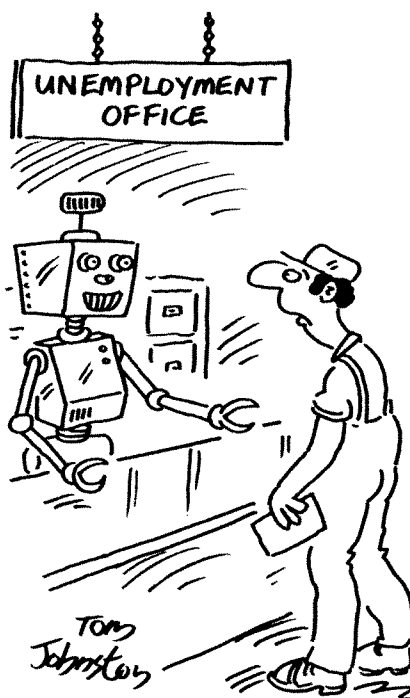
Such automation seems likely to result in a "substantial permanent sector of unemployment", as a result of society's inability to cope with the effects of new technology, predicts Robert T. Lund, a senior research associate at MIT's Centre for Policy Alternatives. Part of the problem, he suggests, is that the 1950s automation scare which failed to materialise has led to a false sense of security.

Some indication of the size of the problem may be given when the Office of Technology Assessment produces the results of a survey it is conducting as part of a broader project on the social impact of data processing systems, with a view to seeing whether any Congressional action may be needed. "It is certainly an issue that deserves more attention than it has been getting," says Mr Stephen Doyle, group manager for telecommunications at OTA.

Meanwhile there is discontent among those who are at the receiving end of the new technology. A survey conducted by the University of Michigan has just reported that, for the first time, there was a decline in the national level of job satisfaction, as measured between 1973 and 1977. The survey of 1,515 workers found that 36% felt their skills were underused, and 32% that they were "overeducated". There was a "slight but significant" drop in overall job satisfaction.

The unions are already beginning to react, largely in response to rank-and-file pressure. In addition to next week's WA demonstration, members of the United Auto Workers made strong demands at a recent conference for tougher terms on retraining, monitoring technical change and giving early notice of automation plans.

Mr Glenn E. Watts, CWA president, goes further. In a recent article he said that his union believes major technological advances should be held in check until a human impact study has been made, just as environmental impact studies are carried out in other circumstances. "If some workers today are bored and alienated, the degrading of work itself must be recognised as part of the problem." □





## FDA backs off attempt to control private DNA research

THE US Food and Drug Administration has concluded that it probably lacks the legal authority to regulate recombinant DNA research in private industry, as it had previously announced its intention to do following a directive from Health Secretary Joseph A Califano last December.

Instead, the FDA plans to encourage companies to comply voluntarily with the guidelines that have been established by the National Institutes of Health for federally-funded research. The NIH is itself recommending some amendments to the guidelines to make them more acceptable to industry.

Two weeks ago, however, the NIH's Recombinant DNA Advisory Committee passed a resolution recommending that private industry be legally required to observe the guidelines. It is now up to Mr Califano to decide whether a scheme of voluntary compliance is adequate, or whether to seek another route for regulation—possibly involving a new legislative initiative.

Following the failure of numerous attempts in the past to extend the NIH guidelines to industry, Dr Fredrickson, director of NIH, announced last year that companies would be able to register with the NIH—if they wished—to have containment levels for proposed experiments approved.

So far, however, companies have been reluctant to register, fearing that compliance with some sections of the guidelines would require them to divulge commercially-sensitive information. At least one company—Genentech, in San Francisco—is now conducting experiments with 60 litres of culture which would have required submitting full details to the RAC for a special exemption from prohibition under current NIH procedures.

The NIH is now proposing to add a new section to the guidelines to meet

such fears. For example, although academic scientists wishing to have a new host-vector system approved by the director of NIH make available full details of the system and its proposed use, this would be waived for private companies, who say it could require disclosing commercial secrets.

Under the proposed addition to the guidelines, private companies would set up their own institutional biohazard committees. But members of such committees would be exempt from the requirement that they should not be involved in approving a project in which they had a financial interest.

The NIH has also gone to some lengths to establish that it would be a criminal act for any member of the RAC, as "temporary government employees", to divulge confidential commercial information submitted to the committee, and that this information would therefore be immune to the Freedom of Information Act.

At a meeting with HEW general counsel Mr Peter Libassi last week, industry representatives said they were reasonably happy with the new proposals as a basis for voluntary compliance. Public interest and labour representatives, however, were adamant that a voluntary system would not work and that some form of legal back-up was necessary. They also urged that the Environmental Protection Agency and the Occupational Safety and Health Administration take a more active role.

The position on legal controls has been complicated by the FDA's decision. Last December, coinciding with the publication of a revised version of the guidelines and following a directive from Mr Califano, the FDA published a "notice of intent" to introduce regulations. These would require a firm submitting a product



*Califano: he must decide*

for licensing by the FDA to demonstrate that any recombinant DNA research carried out in connection with the product had been done in accordance with the NIH guidelines.

Pharmaceutical companies, however, challenged whether the FDA had the authority to do this under the terms of the federal Food Drug and Cosmetic Act, claiming that the agency's authority was restricted to clinical trials and marketing.

Dr C. W. Pettinga, executive vice-president of Eli Lilly and Company, commented: "In our view the proposal must be carefully evaluated because it could evolve into the regulation of basic research, an undesirable circumstance from a public policy standpoint and clearly not warranted in view of the experience with recombinant DNA technology."

No public reply has yet been made by the FDA. But in the light of these and similar comments, officials in the agency confirmed last week that the proposal to introduce new regulations had been dropped and that support was being recommended for the voluntary compliance scheme as being proposed by the NIH.

Dr Fredrickson is now expected to forward these comments, as well as both the NIH's proposals and the RAC resolution, to Mr Califano—who will have to decide how to take things from there.

**David Dickson**

## Plan for early warning on social effects of biomedical advances

THE National Commission for the Protection of Human Subjects, a body set up in 1974 to study the ethical, social and legal implications of advances in biomedical and behavioural research and technology, has recommended setting up an advisory commission "to anticipate the probable effects of research and technological advances for individuals and society, and to stimulate public participation in decision-making".

This recommendation has arisen from a special study conducted by the commission, whose conclusions were published in the *Federal Register* last

week. The commission says that the results of the study showed both a perceived need for a programme to assess the social impact of technology, and a need to facilitate public information and public participation in research and technological innovations and the policy decisions that result.

"These findings suggest that a mechanism should be established to monitor and evaluate innovations and to provide an early warning system in which the probable effect of innovations in biomedical and behavioural research and technology can be assessed publicly, prior to develop-

ment of widespread dissemination", the commission says. Existing entities, such as those attached to the National Academy of Sciences or the National Institutes of Health, served narrower constituencies and goals, and the independence and broader mandate of a new body were needed, it says.

"The commission should not be dominated by health professionals, for its main purpose would be to facilitate widespread debate involving all segments of society in the ethical and policy issues that affect all people and about which diverse views should be heard." □



# California report pinpoints hazards in layout of Three Mile Island control room

In early May, California's governor Edmund G. Brown Jr called for a national moratorium on construction of nuclear power plants and declared himself to be "at the forefront of the antinuclear movement". Some political observers have suggested that his actions represent a new expression of his presidential ambitions, but the governor also seems to be taking seriously a report prepared by his staff that condemns the state of 'human factors engineering' in the nuclear industry. In a letter to Nuclear Regulatory Commission (NRC) chairman Joseph M. Hendrie, Brown wrote: "I am informed that the design of nuclear power plant control rooms may represent a significant safety hazard".

A copy of this staff report, together with some of the supporting documentation, has become available to *Nature*. The report was prepared by Wilson Clark, Assistant to the Governor for Issues and Planning, and three assistants, two of whom have experience in nuclear engineering. It concludes that many nuclear power plants "are poorly instrumented and designed for adequate reactor operator control . . . Modern nuclear technology is harnessed by antiquated control technology which represents a clear and present hazard."

Clark summarised the report's conclusions in a letter to the chairman of the Presidential Commission on the Accident at Three Mile Island, John G. Kemeny. He cited the NRC's preliminary evaluation of the Three Mile Island accident as a vindication of his belief that poor design for human operation were "a leading, if not the leading factor, in the near-disaster at Three Mile Island." He catalogued three main hazards covered in his report that bear on Three Mile Island:

- poor layout, making plant operation difficult and dangerous;
- inadequate display of control room information; and
- confusing and incomplete operational procedures which lengthen operator responses, decreasing reliability.

Clark's point has been made before, but the issue generated little interest until the series of interrelated human and instrumentation errors at Three Mile Island. The famous Reactor Safety Study, WASH-1400, which gave the nuclear industry a generally clean bill of health, criticised the design of controls and displays and their arrangement on operator panels. Later, a task force of the Electric Power Research Institute (EPRI) expressed similar criticism and recommended that EPRI review the problem. The review was

conducted by the Lockheed Missiles and Space Company and published in late 1976. Although the conclusions of the Lockheed/EPRI report were generally low key, the illustrations were devastating. With such understatement as "the study findings paint a rather negative picture" the report catalogues a substantial list of design faults that could directly affect an operator's ability to react quickly in an emergency. These include:

- massive arrays of identical control/display units with no clearly identified subpanel grouping;
- control levers so large that individual control panels expand to enormous size;
- separation of related panels into two areas so that two operators are required to coordinate their actions even when separated by as much as 50 feet; and
- placement of gauges so high that an operator cannot read them without standing on a footstool, or on a wall opposite the location of related control levers.

Even such a simple matter as the number, placement and ease of replacing signal lamps can have serious consequences. The Lockheed/EPRI study found that "indicator reliability is a problem and there are a surprising number of burned-out, single lamp indicators at any given time" in the reactors surveyed. Warning lights are so numerous that many operators become cavalier about 'nuisance alarms'. And to replace burned-out lamps operators sometimes stand precariously on a control panel.

The Clark report shows how prophetic this passage of the 1976 study was to be. In March 1978, an operator dropped a light bulb into an open con-

trol panel at California's Rancho Seco plant, while trying to fix a burned out indicator. The resulting short circuit caused the reactor to shut down, knocked out two-thirds of its temperature, pressure, flow and level signals, and cut off auxiliary feedwater for seven minutes. Clark concludes: "Had other safety systems not performed adequately, this transient could have been similar to the accident at Three Mile Island, which experienced a loss of auxiliary feedwater cooling for only eight minutes."

Governor Brown's team also cited a study of the Zion nuclear power plant by Alan Swain in 1975, for the NRC. According to the Clark report, the owner of the Zion plant had been able to suppress distribution of the document, which had found in a 'talk-through' of procedures to use in a serious accident, that "even experienced operators had some difficulty in locating particular controls and displays."

It remains to be seen what action will be taken to improve the confusing array of instruments, dials and switches facing reactor operators, but the NRC has at least acknowledged the problem. A recent staff report on reactors designed by Babcock and Wilcox (who designed the plant at Three Mile Island) states: "Human factors engineering has not been sufficiently emphasised in the design and layout of the control rooms. The location of instruments and controls in many power plants often increases the likelihood of operator error or, at the least, impedes the operator in efficiently carrying out the normal, abnormal, and emergency actions required of him."

John Douglas

## US to study health effects of accident

THE US Department of Health, Education and Welfare announced last week that it is to carry out a number of surveys of the possible health effects on those who were exposed to radiation as a result of the nuclear accident two months ago at the Three Mile Island nuclear power plant in Pennsylvania.

One survey will cover pregnant women living within 10 miles of the plant, who will receive regular health checks throughout their pregnancy. Their children will be monitored for the first few months after birth. The National Institute of Mental Health will carry out a survey of a representative group of people "subject to the strain of the accident", and a

further study will examine the health of the workers at the plant.

In addition there will be a general survey of the health of 50,000 residents living within five miles of the plant. "It's just common sense to do this. There is no reason for alarm. I said some time ago that we would study the population, and that we would particular focus on pregnant women", Health Secretary Joseph Califano said last week. The survey will be financed by the Center for Disease Control and the National Cancer Institute. State officials hope to continue the study on a year-to-year basis for up to 20 years.

David Dickson



## Support grows for rival conference on development

SEVERAL hundred non-governmental organisations have already expressed interest in participating in an alternative conference which is being planned to take place in parallel with the United Nations Conference on Science and Technology for Development in Vienna at the end of August.

Unlike the official conference, which will concentrate on political mechanisms for stimulating the contributions of science and technology to development, the alternative conference will consider specific topics for action. High on the preliminary agenda are plenary sessions on nuclear energy in Third World countries, environmental aspects of development, the role of multinational corporations, and the implications of the arms race. These sessions will take place in Vienna's Kongresshaus.

A number of working groups are also planned, to run for the full ten days of the conference (August 19 to 29) with the aim of producing specific proposals for action. In particular, there will be discussions about information and financial mechanisms that might be established to support the work of non-governmental organisations after the conference. "This is one of the most important things that we can do at Vienna", says Dr Karim Ahamed of the Natural Resources Defense Council in New York, chairman of the conference organising committee.

"We want to see the extent to which non-governmental organisations can, for example, stimulate technology assessment activities in Third World countries which could be brought to bear on government efforts. And we also want to find out what private and public sources of funding are available to support these efforts."

The organising committee, which has a planning board made up of 14 international organisations, is already preparing an extensive document commenting on the proposals for action that will be discussed at the official conference, and pointing out areas—such as the need for appropriate technologies, or the role of women in development—which it feels have been neglected in official discussions.

"One particular goal will be to see if we can create mechanisms for monitoring what happens after UNCTSD, and exposing it to public view in an effort to make governments keep up to their promises," Mr Ward Morehouse, another member of the committee, said. □

## Max Planck Society sets up three new research institutes—and cuts off support for others

THE Max Planck Gesellschaft (MPG), which spends DM750 million a year on research in West Germany, has started to renew and restructure several of its 50 institutes. It is following a tradition of, from time to time, taking up new fields in exchange for others that have matured or have not developed according to the MPG's expectations.

Last March, the MPG Senate, for the first time in five years, approved the setting up of new institutes (MPI) and gave the green light for three of them straightaway: an MPI of Quantum Optics (Directors: Professors Karl-Ludwig Kompa, Herbert Walter and Siegbert Witkowski); an MPI of Psycholinguistics (Director: Professor Dr Willem J. M. Levelt); and an MPI of International and Foreign Social Legislation (Director: Professor Dr Hans F. Zacher).

In addition, one or two project groups working closely with clinical establishments (eg in universities) are to be set up to augment medical research within MPG. The content and orientation of their research is not yet decided, but it is likely that cancer research will be among the new fields.

The decisions to set up the three new institutes have been taken after several years of successful work by project groups. (This is an important condition within the MPG for the establishment of a new institute.) The new MPI of Quantum Optics is building on the work of the project group for laser research, which itself was hived off four years ago from the MPI of Plasma Physics (IPP). And the future Institute of Psycholinguistics, which will research the structure and use of natural language is building on work already done at the Netherlands University of Nijmegen. The decision to site the latter at Nijmegen is seen as an example of MPG's emphasis on European cooperation. The MPG, which is financed jointly by the federal research ministry and the *Länder*, hopes to fund its new activities from higher growth rates in the budget (6–8% per annum instead of an average of about 3% during recent years of stagnation).

The MPG has also decided to close down work at two of its institutes. Terminating work in a particular field or closing an entire institute is usually done when similar research is being carried out in universities and other research establishments (and mostly when the head of the institute retires).

Thus Professor Jürgen Aschoff's research at the MPI of Behavioural Physiology runs out with his retirement because biorhythms are being researched simultaneously at other places. (His famous 'bunker' will continue to be available to researchers!) For similar reasons the work on nuclear physics at the MPI of Chemistry in Mainz is to run out and be replaced by the new main field of geochemistry.

On average, over the past 10 years, one institute or department within the MPG has been closed or transferred to another agency each year. In the majority of cases the public has been aware of little of the concomitant tensions or problems. Not so, however, in the case of the institute founded in 1970 by Carl Friedrich von Weizsäcker in Starnberg with the ambitious title of Max Planck Institute of Research into the Living Conditions of the Scientific-Technological World. The founding of the institute created a stir in particular because of the all-embracing formulation of its brief (which prompted *Der Spiegel* on one occasion to talk of a 'Faustian' project); the overt attempt by industrialists to influence its policy; and the appointment of the neo-Marxist Jürgen Habermas as co-director.

Doubts later grew about the efficiency of the institute, which seemed to be concentrating on isolated projects. Even though one or two noteworthy pieces of work were done, the commissions, set up as a rule four years before the retirement of an institute head, started to discuss the end of the institute in its existing form. After intensive discussions the MPG Senate decided not to continue the work of Herr von Weizsäcker after his retirement "since there is no one to replace him".

Instead, a new MPI of Social Sciences is being set up, which will have four departments and according to Professor Reimar Lüst, President of the MPG, who was talking during the MPG's general meeting in Mainz last month, "is advancing no sweeping claim for the social sciences, any more than would an Institute of Physics or Biology in our society". Ralf Dahrendorf, at present Director of the London School of Economics and Political Science, was appointed as an additional director with Jürgen Habermas. Two more departments are envisaged for a political scientist and a psychologist.

Klaus Höpfner

# Soviet scientists visit UK and 'sell' technology

"SCIENCE is universal because it strives after universal truths that can be universally verified. Scientists have no option therefore but to collaborate and have done so successfully even when political relations are bad. But technology is different; because it is 'sensitive'."

So said Professor J. Ashworth, Senior Scientific Adviser to the Cabinet Office at the opening last week of the "Days of Soviet Science and Technology" organised in association with the USSR National Exhibition at Earl's Court, London. By this criterion, it might be deduced, both from the exhibition itself and from the composition of the scientific 'touring team', that détente is alive and flourishing. For both are strongly orientated towards technology.

For the exhibition, this may be inevitable; a Soyuz space-capsule or non-polluting oil refinery is much easier to demonstrate to the public than the latest developments in quantum mechanics. Even the announced "massive" participation of the Soviet Academy of Sciences, apart of course for the baby mammoth, reduces to a few technological exhibits—some special industrial alloys, a method of shaping high-precision tools from graphite and then converting them to diamond, and a laser landing system for aircraft which, Academician Basov, the leader of the scientific delegation urged, would be particularly suitable in the British climate.

Basov, who holds a Nobel Prize in physics for his work on lasers, has become, in the context of this tour, virtually a salesman for Soviet laser technology. Even addressing the Royal Society, his subject matter was more practical than theoretical—laser welding, laser precision systems for wire manufacture, laser coagulation for eye-surgery, a laser-based 2,000-line TV set in the "nearest future", as well as the prospects of lasers in the  $10^3$ – $10^6$  J range and a "very promising" hybrid reactor with a "thermonuclear target surrounded by fissile material". The main problem with the latter, he said, will be the "service life but ideologically we are prepared for further developments".

Equally a "salesman" but with longer aims in view is Professor Andrei Kapitsa, whose speciality is remote sensing from space. This he sees as a major field of potential cooperation, particularly in "global problems" such as pollution. "Even a country as large as the Soviet Union cannot save the problem of air pollution", he said. "This can only be done on an international basis." Then, too, there are problems of induced climatic change.

A major irrigation or rain-making initiative by one country could have disastrous consequences for another, if there is no international consultation. According to Kapitsa, however, Soviet planners "don't yet use space data directly, but only at the second or third remove." Science, he said, "is always two steps ahead, giving the economy a tug!"

The "Days of Soviet Science and Technology" are presumably designed to give a similar "tug" to British-Soviet economic cooperation. "Economic cooperation goes very closely with such things as know-how", Kapitsa explained. "Know-how is sold, and know-how is science".

Several members of the delegation, however, saw the "Days" rather as a useful chance of pursuing their own research. The three Medical Academicians, Kochetkov, Loginov and Puchkovskaya all spoke in terms of existing cooperation. So did Academician I. M. Kolotyrkin, who explained that in his particular field—metal corrosion—the UK and USSR have already achieved a *de facto* division of labour. "Britain is doing some very interesting work on the metallurgical side", he said, "stress corrosion, cracking and so on. In the Soviet Union we use mainly a chemical and radiochemical approach. This is a genuine sharing of effort".

For Professor F. V. Sapozhnikov, however, diverging priorities between the UK and the USSR might well have made fruitful discussions difficult. For as Deputy Minister of Power and Electrification, he is committed to a major nuclear power programme, which includes not only power generation, but even the production of hot water for district heating from small reactors sited on the outskirts of cities. (It is considered sufficient for

safety if a zone 3 km in radius round the reactor is kept free from residential use, although it may be used for agriculture, industry or leisure purposes).

The commitment to nuclear energy was well represented at Earl's Court; exhibits included a scale model of the BN-600 water-water reactor intended for the third set of the Beloyarsk power station and a diorama of the South Ukrainian power complex, which will be based on  $4 \times 1,000$  MW nuclear generating sets, with a 1,800 MW hydroelectric auxiliary plant for peak hours and a 380 MW pumped storage system.

Sapozhnikov did not see Britain's current disenchantment with nuclear power as any barrier to cooperation. "We pay a great deal of attention to UK experience", he said. "We have enjoyed a good cooperation programme for several years now, and it has worked quite successfully".

Such remonstrances have in general been fairly infrequent. Apart from the constant stress that exchange benefits the UK as much as it does the Soviet Union, there has been little emphasis on "misunderstandings" and much on mutual benefits and the building of what Basov called "a bridge of confidence" between Soviet and British scientists.

"Our main task is to learn what others have done and to show what we have done", Basov explained. Then, doubtless not unaware of the possé of demonstrators who had dogged the delegation with their banners demanding the release of Orlov, Shcharanskii and Kovalev, he added, "We respect and treat with understanding the customs of your country. As the Russian proverb says 'When you arrive at a strange monastery, don't try to induce your own rules there';"

Vera Rich

## Soviet progress in wind and solar power

SOVIET solar energy enthusiasts may one day give a new meaning to the term industrial plant. Recently Dimitri Zhimerin, Deputy Chairman of the USSR State Committee for Science and Technology, said that "scientists believe that solar energy could be used for industrial applications if generators were developed imitating photosynthesis". So far, however, no-one has announced artificial photosynthesis as a viable method.

Zhimerin's statement, however, came in an otherwise factual account of research into solar and wind power in the Central Asian Steppes. In addition to solar heating for domestic use considerable progress, he said, has been

made in water desalination for state cattle farms. Here the breakeven point occurs when water would otherwise have to travel 35–40 km.

Extensive use of solar energy in these regions could, he said, save the Soviet economy 15–20 million tonnes of conventional fuel per year while wind energy, properly exploited, could produce  $11 \times 10^6$  MW, 50 times the total capacity of all Soviet power stations in 1976. According to Zhimerin, the first wind powered electrical generator with a capacity of 100 kW was built in the Soviet Union in 1930. The experience gained with it has led to the development of "wind powered units, both mechanical and electrical". □

**CO<sub>2</sub> increased by 1.5 p.p.m. in atmosphere last year:** The US National Oceanic and Atmospheric Administration reported that the average carbon dioxide concentrations in the earth's atmosphere rose to 335 parts per million last year, an increase of 1.5 parts per million from 1977, and of 21 parts per million—or 6.3%—since 1958. A spokesman for the agency said that the increase was principally due to increasing emissions of carbon dioxide from the burning of fossil fuel. Despite the increases, however, government scientists still believe that they have several years in which to decide whether the effects of the carbon dioxide build-up warrant restrictions on the burning of coal, to which the US is increasingly turning as an alternative fuel to oil.

**Iran says no to nuclear power:** The revolutionary government in Iran has cancelled all plans for nuclear power plants in the country including two contracts for plants already under construction. The head of Iran's atomic energy organisation, Fereidun Sahabi, said the contracts with France and Germany should be cancelled for "political, economic, social, human and technical reasons". The German plant is 80% complete but overspending of £1.5bn would mean that the final cost of £3.5bn for the plant would be more than twice the international standard. "It is true that work conditions in Iran are different than in Germany, but they are not that different", said Sahabi. An Iranian energy expert, formerly in favour of nuclear energy, has prepared an analysis showing that the plants cooling towers could be used as grain silos. The nuclear cancellations are only part of a massive reorganisation of Iran's economy.

The *Financial Times* estimates that multinational losses in Iran amount to £18.6bn in the civilian sector with an equal amount in the military sector, a level of loss "unprecedented in business world wide short of a natural disaster or a global war". US and UK firms have been hardest hit because of their heavy involvement in the sale of military equipment to the Shah. The rise of workers committees in the factories has been instrumental in the cancellation of many contracts.

**Meteorologists approve world climate programme:** The executive board of the World Meteorological Organisation at its 8th World Congress on 26 May passed a basic programme of research into the world's climate. The programme will collate data from WMO's World Weather Watch initiated eight years ago and data acquired from the climate programmes of member countries in order to assess climatic change and variability with special reference to predictability and human interference. This study will be carried with the International Council of Scientific Unions. In addition, a study of the impact of climate on human activities will be carried out in collaboration with the United Nations Environment Programme. Two other projects will be a new typhoon operational experiment in the Western Pacific with special attention to hydrological effects inland and a West African monsoon experiment. There will also be an increased emphasis on training programmes in Third World countries to be carried out with money from the UN Development Programme.

*from Peter Collins in Geneva.*

**USSR asks US to halt space shuttle programme:** The Soviet Union has asked the US to halt work on the space shuttle programme, which it sees as a possible threat to its own satellites, according to a report which appeared last week in the *New York Times*. The demand is said to

have come up during preliminary discussions on ways of eliminating the spread of so-called killer satellites, which are designed to locate and destroy other craft in orbit, such as surveillance and communications satellites.

The space shuttle, which has been developed by the National Aeronautics Administration for both civilian and military purposes, is due to be launched at the end of the year. It will be used to launch military satellites into orbit, but US officials deny that there is any intention of using it for anti-satellite activities. The Soviet Union has maintained, however, that the shuttle could threaten its satellites, and has therefore asked the US to halt the testing programme. US officials describe the request as "totally unacceptable" and feel that it rules out the possibility of an early agreement on killer satellites, which they had hoped to reach before the signing of the SALT treaty by President Carter and Mr Brezhnev in Vienna on 18 June.

**Hundreds arrested, one killed in worldwide nuclear protests:** A weekend of militant anti-nuclear activity was marred by the killing in Spain of Ladi de Estan Terrane, 24, who was shot in the head during a police charge on an anti-nuclear rally in Tudela in the Basque country. The killing provoked three hours of street fighting between demonstrators and police. Anti-nuclear demonstrations also drew large numbers of people in the Netherlands, Japan, the UK and West Germany. In Canada, five sky-divers jumped into forbidden territory at the world's largest nuclear plant under construction in Darlington, Ontario and 56 others were arrested at the plant owned by Ontario Hydro. In the US over 600 people were arrested in demonstrations at plants in 12 American states. In Inola, Oklahoma 339 demonstrators braved a biting rain to get arrested by climbing the fence guarding the construction site of the Black Fox nuclear power plant.

**Test ban critic to head weapons research laboratory:** The University of California announced last week that Dr Donald M. Kerr, currently a deputy assistant secretary in the Department of Energy, has been appointed the new director of the Los Alamos Scientific Laboratory in New Mexico, succeeding Dr Harold Agnew who resigned earlier this year. Dr Kerr worked at Los Alamos, which carried out a large proportion of the US basic research into new nuclear weapons, for ten years before moving to Washington. He became the focus of some controversy last year when he opposed President Carter's proposals for a five-year test ban treaty, claiming that a treaty of such length would raise questions about the reliability of weapons that were being stockpiled but not tested.

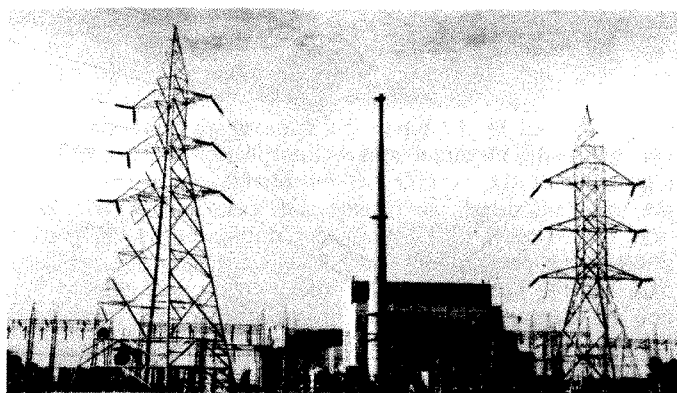
Mr Kerr's previous involvement in weapons research is likely to lead to renewed pressures at the University of California for the university to divest itself of the Los Alamos and Lawrence Livermore laboratories, which it manages on a contract basis for the Department of Energy. Last month, an advisory committee recommended to the Department of Energy that it should prepare contingency plans, in case the university pulls out.

**Dungeness okay:** The UK Health and Safety Executive has permitted the Central Electricity Generating Board to restart its Dungeness nuclear reactor. The reactor was shut down when micro-cracks were discovered in the welds of an expansion bellows. The CEBG claimed that the cracks were present at manufacture, have not increased during use and are not a hazard. Monitoring equipment has been installed as an additional precaution.

# This nuclear power plant is contaminated.

## India's demand for energy means it cannot be shut down

*Tarapur, near Bombay.*



Nuclear power needs to be regulated in the Third World just as much as in the West. But economic pressures in developing countries mean that safety may not always come first. Moreover, western insistence on regulation may be seen as an attempt to hamper Third World progress. Report by **Anil Agarwal**

THE accident at the Three Mile Island nuclear power plant has caused considerable concern in the developed countries. But very little has yet been said about the lessons to be learnt on the safety of nuclear reactors in the Third World. Even though volumes have been written about the problems of transferring sophisticated technology to developing countries, precious little has been published about the management and absorption problems posed specifically by the transfer of nuclear technologies to the Third World. The sole exception in this field is the burgeoning literature on the proliferation aspects of nuclear technology transfer.

In the past few years, rising costs and environmental protests have halted the growth of nuclear programmes in western countries. In 1977 almost no new reactors were ordered by the industrialised countries. During this time, it seemed as if the nuclear industry had begun to shift all its attention to Third World markets in particular, with French and West German companies indulging in aggressive selling openly aided by their governments. The prospects of large orders from Brazil, Iran and South Korea seemed to confirm this trend.

Since the revolution in Iran, however, Third World markets have received a serious blow. Iran has cancelled all the nuclear plants on order and according to unconfirmed reports, Iranian authorities—and for that matter, the Western nuclear suppliers too—may not be interested in completing the Bushehr nuclear power

plant which has reached an advanced stage of construction. Among the first set of people arrested by the Shah of Iran for mismanagement and embezzlement in his unsuccessful bid to satisfy the rising demands of his people was the then Chairman of the Atomic Energy Organisation of Iran. Since the overthrow of the Shah's government, several officials of the AEOI have left Iran leaving behind only a fledgling organisation.

Brazil's controversial nuclear programme too is running up against increasing domestic criticism largely because of escalating costs. The cost of Brazil's first power reactor, the 626 MW Angra-1, which is being constructed by Westinghouse, is now expected to be between \$850 million and \$1 billion compared to the initial estimates of \$218 million. The cost of the Brazilian-West German nuclear deal of 1975 under which Brazil was to be supplied with eight nuclear power plants of 1,320 MW each by the West German company Kraftwerk Union (KWU), has already risen from \$10 billion to \$13 billion. Industry is protesting at the projected increases in electricity tariffs. Meanwhile, US pressures have prevented the French sale of a plutonium reprocessing plant to Pakistan.

Third World nuclear programmes are, therefore, slowing down under a variety of economic and political pressures. But several developing countries nonetheless have or are planning to have significant nuclear programmes. South Korea ordered two new plants in 1978 and already has one reactor in operation, and two under construction. By the mid-1980s it expects to have eight nuclear power plants that will supply nearly one-third of its electrical needs. In nuclear terms, therefore, South Korea should become Asia's second Japan. Taiwan too is pressing ahead with its nuclear programme despite the supply problems posed by its current political difficulties. Taiwan's first power station consisting of two 636 MW units started operation in 1977 and two more large plants are expected to go into service

by the early 1980s.

The Pakistan Atomic Energy Commission has asked its government to sanction the purchase of a 600 MW nuclear plant. Turkey is expected to order its first plant from Sweden soon. Argentina already has one 319 MW power reactor in operation and another 600 MW reactor under construction. It is also expected to decide on the purchase of more reactors soon, possibly this month. Argentina is mainly considering tenders from Canada and Germany. Cuba plans to go ahead with a 440 MW nuclear reactor from USSR. Mexico has two reactors under construction which are expected to be completed by 1982. With the discovery of large oil reserves, Mexico may not be keen to go ahead with more reactors in the near future.

From all present indications, it seems that the incident of Three Mile Island has scarcely sent a ripple through those Third World countries which are keen to buy and build as many nuclear reactors as they can. Part of the reason for this behaviour is that nuclear programmes have come to be associated by developing countries with enormous political prestige. The efforts of western governments to control the spread of nuclear technologies are seen by many Third World governments as a crude attempt to monopolise a technology that is of considerable importance to the world. These discriminatory western pressures have helped to make nuclear power, as a senior IAEA official recently put it, "an immensely patriotic issue" in many developing countries and even in some developed ones like Japan. Under these circumstances, nuclear authorities in Third World countries will move very cautiously to accept safety-related arguments against nuclear power.

However, where safety *per se* may fail to move Third World nuclear authorities to ensure safest possible operation of nuclear projects, sheer economics will force them to consider all safety-related issues. Unlike the US, no developing country can suddenly afford to lose a billion dollar plant because of an accident. In some



countries, a large power plant can form as much as 10% of the electrical grid. The loss of such a plant could plunge the country into an energy crisis that could cripple its growing industry.

Once a developing country has an unsafe plant therefore these economic pressures could persuade it to continue dangerous operations. The Tarapur Atomic Power Station (TAPS) on the western coast of India which supplies power to the industrially important metropolis of Bombay can, for instance, be placed in such a category. TAPS was the first atomic power station to go in operation in the Third World. It was built on a turnkey basis by General Electric under an Indo-US agreement and commissioned in 1969. Defective fuel bundles supplied by General Electric led to widespread contamination of the plant and extremely high radioactivity levels. *Business India*, an Indian journal, recently reported in an extensive survey of India's nuclear programme: "TAPS is so heavily contaminated . . . that it is impossible for maintenance jobs to be performed without the maintenance personnel exceeding the fortnightly dose of 400 mrem in a matter of minutes. Thus the maintenance worker—who is often not an employee of TAPS—holding a spanner in one hand and a pencil dosimeter in the other, turning a nut two, three rotations and rushing out of the work area is a common phenomenon in TAPS." When asked why not shut down TAPS and decontaminate it thoroughly, a senior TAPS engineer replied: "Ideally, that should have been done in 1974 or earlier but there is such great pressure from the Department of Atomic Energy on us to produce power that we cannot shut down."

It must, however, be said to the credit of the Indian nuclear authorities, who control the best technical expertise in the nuclear field in the Third World, that they have taken considerable care to minimise the radioactivity problems in TAPS. India has a massive nuclear R&D programme which accounted for nearly one-fifth of the India government's R&D budget of Rs 3,200 million (about £220 million) in 1976-77. Despite this indigenous capability, Indian engineers had to seek the assistance of General Electric when fuel defects and related radioactivity problems first began to surface—and they obtained very little help. General Electric simply blamed them for mismanagement and lack of technical expertise. Even the US Atomic Energy Commission officials were then forced to prepare a note for Commissioner James T. Ramey in which they stated: "US manufacturers have extremely poor records of information disclosure to foreign

purchasers. Designs at times do not reflect all the regulatory items required in the US; at times design innovations are first tried by US manufacturers in overseas reactors. US manufacturers normally deal with foreign utility companies and short-cut their assistance to foreign governmental control groups (like nuclear regulatory agencies), which are sometimes in weak governmental positions." These discussions led Commissioner Ramey to emphasise that General Electric should be very careful with the nuclear plant it is setting up in Mexico. Mr Ramey pointed out to General Electric that "Mexico will have no technical back-up such as that which is available in India at the Bhabha Atomic Research Centre, and that GE should pay particular attention to assuring there is an adequate operations and maintenance staff, whether or not they have a contractual obligation to do so."

Dr Morris Rosen of the Nuclear Safety Section of the International Atomic Energy Agency has further listed several critical aspects of nuclear power plant exports to developing countries. At the time of the IAEA-sponsored International Conference on Nuclear Power held in Salzburg, Rosen pointed out that several power plants quite dissimilar to those that exist in supplier countries are being built in developing countries. For instance, he revealed that high seismicity at the site of two large reactors sold by West German vendors to Iran could result "in significant design changes in the nuclear facility; changes that influence the foundations, interface of structures, pipe requirements, supports and system components (including reactor internals). Thus, the eventual design of the Iranian facility as constructed may have some significant differences from modifications which will not have

undergone a detailed review by the regulatory bodies of the Federal Republic of Germany."

Rosen pointed out another example for which there was no reference plant—a similarly sized plant under construction in the exporting country which meets its safety requirements and therefore can be licensed. "The recent 2-loop reactor plant for Egypt, Korea, and the Philippines, is referenced to a 2-loop reactor plant under construction in Yugoslavia since 1974. This plant in turn had been referenced to an earlier 2-loop plant under construction in Brazil, which in turn had been referenced to a domestic plant in Puerto Rico. However, the review of the Puerto Rico plant was terminated in late 1972 . . . and it was determined not to continue with the project. If the Puerto Rico plant had been constructed, it would have undergone a systematic and detailed review by the US regulatory organisation and as a result of this review . . . a number of modifications would undoubtedly have been made. Thus, all of the previously mentioned exported 2-loop plants have undergone a rigorous regulatory review, and modifications that might have been required are not available for consideration."

The prime need of developing countries with nuclear programmes is strong and competent regulatory agencies—unfortunately something they very much lack. In the words of Mr Rosen: "At the present time, with little exception, the regulatory organisations of developing countries with active nuclear programmes can be classified as sub-minimal. In many cases they consist of less than 15 full-time staff members associated with nuclear power activities. This minimal staff may not be familiar with the disciplines of nuclear safety and may



South Korea: first steps into nuclear power, 1974.



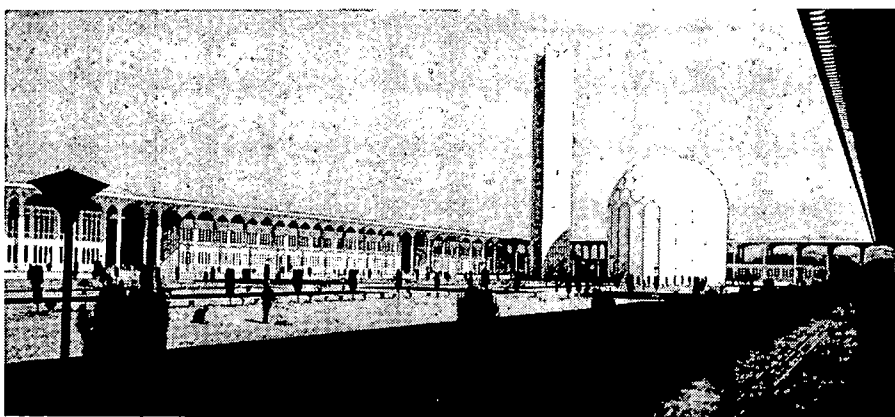
be in need of extensive training."

Without good regulatory agencies, developing countries can neither ensure they are being sold safe nuclear systems or that they are being operated safely. There are several obstacles to the setting up of a strong regulatory agency in a developing country. First of all, nuclear power is such a sensitive issue politically that few governments would risk setting up an organisation whose main responsibility would be to make critical reviews. Second, there is the question of cost which can be relatively very high for a small one- or two-reactor country. Third, there is the lack of technically qualified personnel.

To minimise their expenditures on nuclear programmes, most Third World governments hold the budgets of their regulatory agencies at very low levels both in foreign and local currencies. South Korea is a typical example. The Korean authorities have been making use of foreign consultants for their nuclear regulatory programme. Two separate studies have been completed, including one which gives detailed recommendations for the organisation of the regulatory body. But the South Korean government has still not put up the funds for implementing the recommendations. South Korea has also had an IAEA mission for evaluating site and safety reports. Though such procedures are useful, IAEA officials themselves do not consider them a substitute for a good domestic regulatory organisation. Like those of South Korea, regulatory units in Taiwan, Philippines and Mexico are also very weak.

At an IAEA-sponsored symposium on problems associated with the export of nuclear power plants, representatives of the Mexican Instituto Nacional de Energia Nuclear pointed out that the Mexican "regulatory body is still considered to be working under less than ideal conditions. For example, licensing actions have been taken with insufficient information and the construction schedule is such that in some cases INEN has had to make certain judgements without evaluating all the relevant safety considerations."

The Mexican case is particularly interesting as Mexico obtains its nuclear fuel through the IAEA and therefore agrees to comply with the applicable IAEA safety regulations. IAEA missions have visited the Laguna Verde Nuclear Project and identified several deficiencies. But little action has been taken. The paper quoted above points out: "Subsequent IAEA missions reported that little attention had been paid to previous recommendations. The Agency's Director General issued a second letter to Mexico's Ambassador to the United Nations. This letter was



*Western technology meets eastern architecture: Pakistan's nuclear institute at Islamabad.*

much more critical than the first one; however, the authors are not aware of the response to the letter nor of the actions taken by the Mexican government as a consequence."

The IAEA has a similar agreement with Yugoslavia but there the regulatory body is practically non-existent. A nuclear plant is under construction at Krsko with costs being shared by the republics of Croatia and Slovenia. Neither of them are interested in setting up a regulatory agency, nor are the federal authorities because of the considerable independence granted to the republics under the country's constitution. Perhaps the regulatory situation will improve when Yugoslavia becomes a two-reactor country. In India, there is considerable domestic competence, but the Atomic Energy Commission is both utility and regulator.

The IAEA's Director General, Dr Sigvard Eklund, invited a group of international experts to Vienna at the end of last month to discuss the safety issues thrown up by the Three Mile Island incident and the manner in which the agency should respond to them. The agency is currently preparing under its Nuclear Safety Standards (NUSS) programme, about 50 books in the form of Codes of Practice and Safety Guides. These will certainly help regulatory agencies in the developing countries to insist upon at least minimum requirements for safe operation of nuclear plants. But given weak regulatory bodies they will clearly have a limited impact.

An important activity that the agency could undertake is to send more safety missions to help IAEA member-states and possibly set up a system by which the agency could inspect power reactors. If the member-states of the agency were agreeable, there could be a resolution at the Board of Governors that they will adhere to the safety regulations stipulated by the agency or there could even be a specific international convention.

It is, however, doubtful whether

many Third World governments will react very favourably to such suggestions. They could be easily interpreted as yet another attempt by western governments to control the development of nuclear power in the Third World. A simple compromise like making IAEA recommendations only advisory and not mandatory could be a solution. But if countries lack the political will to ensure maximum safety or possess systems in which the utility is politically far more powerful than the regulatory agency, then regulatory work can turn out to be only a lot of paper work. IAEA safety missions could expect a considerable degree of hostility.

The inspection-related issues are likely to be discussed further at the meeting of the IAEA's Board of Governors later this month. The least that the agency could do is to step up the flow of information amongst member-states about nuclear mishaps which would help to spread understanding about how to cope with them. The Three Mile Island accident did show that proper training of operators is a matter of vital importance.

An activity that the IAEA is planning to undertake soon is the organisation of an emergency assistance system in the case of major nuclear mishaps. This would mean maintaining an international roster of experts on immediate call. The IAEA's nuclear fire-fighting brigade will, however, not be easy to operate. Experts will have to be flown from various parts of the world which will take a considerable amount of valuable time. Nonetheless, such a system will be a step in the right direction. In the TMI disaster, experts were required for several days.

In spite of international actions, the mishap at the Three Mile Island means that Third World governments with nuclear programmes will have to be more concerned with safety—and developed countries will have to ensure that safe technologies are transferred, especially to places where technical skills are still lacking. □

# How Ghana cleaned up on soap but failed with nuts and bolts

In the last of his series, **Joseph Hanlon** looks at African attempts to turn 'appropriate technology' into small industries.

SCIENTISTS and technologists anxious to make a contribution to Third World development have in recent years often turned to 'appropriate technology', where there is a real need for science and engineering input. Several universities have set up AT centres, and one of the most successful—measured in terms of technology actually put into industrial production—is the Technology Consultancy Centre (TCC) at the University of Science and Technology in Kumasi, Ghana. But the TCC's experience shows that despite the need for scientific input, the key questions are not technical. Despite its success by the standards of other AT centres, the TCC is increasingly worried about its lack of impact in Ghana, and is implicitly raising questions about the whole concept of university AT centres.

Windmills always seem to be the first project that AT centres try: they seem such a nice, small, decentralised energy technology—just what the people 'out there' need. But there are no windmills at TCC. "People who have whole research programmes into windmills ask us why we don't have a windmill project. I ask where the application is—no one has ever come and asked us for a windmill," explains Dr John Powell, TCC director. The basis of TCC is that it "very seldom comes up with its own ideas. If you go to people and say 'You should do this', then when you go away nothing happens. But if they come to you, there is a faint chance it may happen."

This philosophy has led directly to the TCC successes. The first came just after the centre began in 1972. A local man, Mr Baffoe, who made laundry starch from cassava, came to the centre to ask about making paper glue. Schools traditionally make their own glue from cassava starch and the ash from plantain peel. (Plantain is a local banana-like food which is very common.) But the glue has a very short life and is not rewettable. Baffoe was passed on to Dr Rao in the university chemistry department, who found a non-toxic fungicide to use as a preservative, and chemicals for rewettability. Baffoe began making the 'spider glue' in the courtyard of his compound, and within a year was a fairly rich man.

The glue is an ideal example of appropriate technology. It is made almost entirely from local materials and replaces an imported product. Baffoe was able to supply most of Ghana's paper

glue needs, saving considerable foreign exchange, and his glue cost less than imported glue on the local market. Furthermore, it was small scale, used labour instead of capital, and it required some scientific input.

TCC's biggest success to date has been with soap making. Soon after the TCC opened, local small scale soap makers came to the TCC asking for help in improving the manufacturing methods. Engineering and chemical analysis produced an improved formula. With £10,000 from the Ghana government, work began on improved soap making tanks. The TCC committee, composed of two engineers, a pharmacist, and a chemist, "wanted a mini Lever Brothers," Powell said, but after much discussion, the committee was talked out of advanced technology and tanks using electric kettle elements were made. They "worked well with professional supervision on the campus. But it proved too sophisticated for rural soapmakers. I would expect that now, but didn't then—it was our first big project."

With help from the Intermediate Technology Development Group (ITDG) in London, G. Prakash, a consultant on small scale soap making at the Appropriate Technology Development Association in Lucknow, India, went to the TCC. He immediately redesigned the tanks to make them easier to control, and converted them for use on wood fires. Not only was it easier for the rural soapmakers to operate, it was a better technology—both capital and operating costs for the wood fired plants were less than half those of the electric plants. The project has been a considerable success, probably the biggest of any university AT project: more than 100 soap making tanks of TCC design have been made.

The soap is produced from palm oil and caustic soda. Within a few months of the first plant opening, however, caustic soda began to run short, and TCC turned to the chemical engineering department for help. There was an acetylene plant in Tema which imported calcium carbide and reacted it with water; the waste product, calcium hydroxide was simply dumped outside the gate. This could be reacted with sodium carbonate, which still has to be imported, but at half the price of the final caustic soda. So a simple electrically heated, mechanically stirred plant was built which produced caustic soda

for less than the market price. Six are now installed. Shortages of oil for soap making led to involvement in oil palm plantations, alternative oil seeds, and improved oil presses.

TCC is now a major operation, with 80 staff and a £100,000 per year budget. More than half that money comes from TCC's own production units, which manufacture soap, nuts and bolts, and equipment such as the soap making tanks.

Its biggest project to date is a pyrolytic converter to make charcoal, gas, and oil from the sawdust available in abundance from the sawmills around Kumasi. If wood is burned by the traditional charcoal burners, the charcoal produced amounts to only 10% of the original weight of the wood. By carefully controlling the burning temperature in the pyrolysis process, it is possible to produce 25% by weight of charcoal, plus 10% oil. No fuel input is required since the output gas is used to dry the sawdust, and the burning wood provides its own heat for pyrolysis. The Ghana Government, USAID, and the Georgia Institute of Technology have all helped in what is a quite sophisticated project—temperature must be carefully controlled, and there are special problems because the density of hardwood dust is different from that of softwoods used in this system elsewhere. Nevertheless, a pilot plant on the UST campus is already processing more than 1 tonne of sawdust a day and has reached 22% charcoal and 7% oil.

From the platform of these successes, Powell and others at TCC are now raising some questions. They point out that appropriate technologies can be surprisingly marginal. For example, there is a controlled price for soap, and when rocketing palm oil prices made it uneconomic to produce soap at the controlled price, the government gave a subsidy to Unilever, but not to small soap makers. Eventually, TCC and other soap makers had simply to ignore the controlled price. Similarly, several TCC projects depend on small imported items such as bearings and colourings that are simply smuggled in because import licences are not available.

Animal feed production from brewers spent grain is a case in point. Grain left over from brewing ferments within a day, and Kumasi Brewery throws away 100 tonnes a week. The Department of Biochemistry at UST analysed the grain for a local entrepreneur and found it suitable as an animal feed (a common use of spent grain in other countries). TCC developed an effective drying process, involving a press and sun-drying, and the entrepreneur was soon in business. The nearby Guinness Brewery bought a highly mechanised drying plant at about the same time.



This runs at a loss, despite charging 6 cedis a bag for dried spent grain, while the local entrepreneur with his simple technology makes a profit at 4 cedis a bag. But he is surviving in business only because the Kumasi Brewery has consistently been refused import licenses for machinery like that used by Guinness. But how long will that continue?

The successes of TCC, however, only make clear its biggest failure. "Contrary to expectation, entrepreneurs have been very slow to follow the lead given by the university," comments vice chancellor E. Bamfo Kwakye, a strong backer of TCC. He thought that entrepreneurs would take things out of their hands, as with soap and glue, but this has not happened. Many products, such as nuts and bolts, are still manufactured on campus. There seem to be three reasons—the location of TCC, the nature of Ghanaian entrepreneurs, and economic factors.

Many small businessmen are unwilling to come to the large university campus outside Kumasi. So TCC has decided to go to them. It proposes two Intermediate Technology Transfer Units in the middle of existing informal industrial areas. These would actually manufacture products, but they would also provide training, designs, and other help to upgrade existing craftsmen. One unit will be in Suame Magazine, Kumasi, Ghana's largest informal industrial area, which has thousands of craftsmen, like fitters and carpenters. The unit there will be involved in blacksmithing, carpentry, and so on, and will have its own machine shop. The second unit will be in Tamale, in the north, and concentrate on the manufacture and repair of agricultural equipment. Funding will come from the US and Canada.

The second problem is a lack of Ghanaian industrial entrepreneurs. Ghana is well known as a trading country. It has large markets, and every family seems to have one member involved in trading or transport. This may be part of the colonial heritage—the British saw Ghana as a source of raw materials and a market for manufactured products, but they were rarely directly involved in the internal economy, as in their other African colonies. Local people were encouraged to trade, but not to manufacture goods which might compete with Britain. Trading requires different skills from manufacture, resulting in a dearth of industrial management expertise in Ghana, and this is one factor cited as a reason to opt for advanced technology, which requires fewer managers than decentralised AT. In any case, Ghanaians find trading much more pleasant—trips and chats are part of the work, the hours are flexible, and



TCC's pyrolytic converter: sawdust is burned in the tank and the gases condensed in a water collar to produce oil. Left is TCC director Dr John Powell.

profits can be very high. Industrial work, on the other hand, is physically hard, much steadier, and often noisy and dirty.

The third problem is economic. As Sally Holtermann, an economist who recently studied TCC for the Intermediate Technology Development Group in London, points out: "in an economy where the activity is to be carried out by private enterprise, entrepreneurs are not going to invest in plant and equipment that is not going to yield them a profit." Sometimes Powell recognises this. But he also told me: "You can get awfully confused with economics. Sometimes I think it is better to avoid economics and concentrate on technology."

The result is best shown by nut and bolt making. Because of import restrictions, craftsmen in Suame Magazine were having trouble getting coach bolts for the wooden bodies they make for passenger lorries. So the TCC imported some used equipment, including capstan lathes, and set up a shop on campus making nuts and bolts. It seemed an obvious technology to transfer—but it hasn't been, for reasons now becoming clear. The market is dependent on import restrictions, since imported nuts and bolts, when they are available, are one-third the price of those made at TCC. Second, making one-off motor spares and machine parts is just as profitable for the entrepreneur with this machinery, and much more interesting than long production runs of bolts. And third, TCC found out only last year that for four years its nut and bolt shop has been losing money. It now makes a profit, but not enough to interest a Ghanaian businessman.

The response of TCC has been to question the attitude of the Ghanaians. "If a nation is ripe for development, it will go forward, no matter what the development technology. India and China have reached this psychological take-off point, but it hasn't happened

here," Powell argues. "The average Ghanaian trader, if he can't make his profit today, isn't interested. Few people here realise that development does not take place overnight—you have to work for your children's children." The answer is not that simple, though. Could it be that Ghanaians are suggesting in practice that although a technology meets academic definitions of 'appropriateness,' it may not be right for Ghana?

TCC has received strong official backing. It was even mentioned in the government's last five year plan. But it has had little contact with government research and development units. It gets strong backing from the university, and practical support from a few people like Kwakye. But there is virtually no participation from other Ghanaians at the university. Nearly all of its work is done by expatriates at the university, or by Ghanaians actually on the TCC staff, some of whom are extremely dedicated. Even Powell mentions the almost complete lack of student interest in TCC. Nor have TCC's successes increased involvement; in fact, the opposite has occurred. After the success with spider glue, many faculty members refused to cooperate because they felt they were being exploited. And at the centre itself, it is the strength of expatriate Powell that keeps TCC running. People may come in at 7.30 each morning when he is there, but they don't when he is on leave.

Powell himself may have the answer. "AT will only work when it is done as in India—by people for themselves. Here, it's not Ghanaian professors working for Ghanaian peasants. The whole thing is an expatriate activity, an oboroni [white man's] hobby. I don't have to use the soap made in my factories. It's technology for them, not for us." But when the other alternative is Unilever, perhaps all Ghana can do is choose between different oboroni technologies. □



# news and views

## Fibronectin: a function at the junction

from Clive Lloyd

THE fact that suspended tissue cells attach and flatten on suitable surfaces is not only experimentally convenient but a powerful example of the trans-action between internal musculature and the external environment. Involvement of the cell's actomyosin system in making contact and generating movement began to emerge some time ago and now there is evidence to promote the involvement of the extracellular molecule, fibronectin (also known as LETS protein), in forming organised cell adhesions. The significance of these recent findings is that fibronectin and associated extracellular proteins—perhaps in a process common to many biological events—stimulate the formation of cytoplasmic organisation. This raises questions about the nature of the transmembrane steering linkage between the two systems.

At the observational level tissue culture cells which are spread on a culture dish become spheroidal when brought into suspension but will flatten once more when restored to some suitable surface. At the molecular level, this cycle of events can be described in terms of the development of the internal musculature by which a cell grips the substratum (Rees *et al.* *Nature* **267**, 124; 1977) and then the loosening of this grip (and hence the loss of asymmetrical cell shape) in the presence of dissociating agents. Components of this actomyosin system can exist in different states of organisational complexity (see Lazarides and Revel, *Sci. Am.* May 1979, for a graphic account) but conspicuous microfilament bundles which characterise and maintain the well-spread cell often seem to 'crystallise' out from localised points of cell-cell or cell-substratum contact. Because not all surfaces support active cell spreading, the nature of the congenial substratum is clearly important in setting up microfilament bundles and this is where fibronectin seems to play a major part. For instance, transformed cells deficient in microfilament bundles,

spreading ability and cell surface fibronectin (LETS) can be converted back to a more normal spread morphology with abundant microfilament bundles by the administration of fibronectin (Willingham *et al.* *Cell* **10**, 375; 1977; Ali *et al.* *Cell* **11**, 115; 1977). Conversely, the dissociating agent trypsin, at low levels, removes LETS from the cell surface (Hynes *Proc. natn. Acad. Sci. U.S.A.* **70**, 3170; 1973), perturbs the organisation of cytoplasmic microfilament bundles and converts well-spread cells into rounded ones (Pollack and Rifkin, *Cell* **6**, 495; 1975). The distribution of fibronectin is consistent with its role in cell attachment for it can occur as a fibrous meshwork sandwiched between the cell and the substratum and is also observed between cells in contact (see Yamada and Olden *Nature* **275**, 179; 1978 for general review).

These general properties are an already accepted chapter of the fibronectin/LETS saga but a more detailed examination of the correspondence between this extracellular molecule and the cytoskeleton will be required before any reasonable molecular explanation of their relationship emerges. Towards such an explanation, Hynes and Destree (*Cell* **15**, 875; 1978) used double-label immunofluorescence to tackle the problem. They show that actin microfilament bundles close to the cell's substratum are often (but not always) co-extensive with fibronectin outside the cell because the rhodamine anti-fibronectin and fluorescein anti-actin image are, to a reasonable degree, superimposable. The resolution of this technique is suggested to be not greater than 200 nm, but still more recent evidence suggests that any transmembrane gap between these parallel systems of fibres (if one exists at all) is of the order of 8–22 nm. I. I. Singer (*Cell* **16**, 675; 1979)

reported electron microscopy of thin sections cut through regions where internal microfilament bundles and external fibronectin-containing fibres meet, proposing that the electron-dense plaque where the two systems come together be called the fibronexus. Sections cut approximately parallel to the plasma membrane gave the appearance of microfilament bundles overshooting the plasma membrane and continuing on through the extracellular space—a conclusion that Perdue (*J. Cell Biol* **58**, 265; 1973) had previously reached by an electron-histochemical study of chick fibroblasts. With the benefit of ferritin-conjugated antibodies to fibronectin (probably not available to Perdue at that time) Singer shows that the extracellular portion of this transcellular highway contains fibronectin. This agrees with Hynes and Destree's observations that "actin microfilament bundles frequently terminated before the periphery of the cell, whereas the co-linear fibronectin often continued further towards the edge of the cell and even beyond it". Singer tilted his sections through 40° in the electron microscope to see if there was any hidden gap between the two systems but, given the limits to resolution, he saw none and the structures remained co-linear throughout. This tends to suggest that where fibronectin and microfilament bundles are related, they are related in a 1:1 manner, but whether this relationship is co-axial or of overlapping ends with some special binding structure in between, is an open question. The fibronexus, then, would seem to be synonymous with cell adhesion plaques except that a role for fibronectin in forming such junctions is now more clearly claimed.

The recent paper of Thom *et al.* (*J. Cell Sci.* **35**, 281; 1979) bears directly on this and concludes that fibronectin is necessary for the formation of organised junctions, but not entirely sufficient. Interference reflection microscopy is a technique that

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produces a contour-map of the under-surface of the cell—the focal points of close contact with the glass ('feet') being darker than more distant areas of the cell body. These 'feet' are known to be the terminations of microfilament bundles (Heath & Dunn *J. Cell Sci.* **29**, 197; 1978) and in the electron microscope they have a structure resembling half of an intercellular junction of the adherens type (see Heaysman, in *Locomotion of Tissue Cells*, Ciba Symp. No. 14, 187; 1973). Thom *et al.* demonstrate that a line of rat fibroblasts attaches and spreads on glass pre-coated with fibronectin isolated from chick serum but that the interference reflection image of 'feet' remains poor and not fully organised unless other components of serum are also present.

Such other components may therefore either directly participate in the organised adhesion or may merely maintain the cell until it can tailor its own fibronectin into more favourable arrangements than occur adsorbed onto pre-coated glass.

As for the internal face of the junction, Singer echoes the view that the fibronexus may be the nucleation centre for actin bundle formation, quoting Lazarides and Burridge's observation (*Cell* **6**, 289; 1975) that the Z-band protein of skeletal muscle ( $\alpha$ -actinin, which holds actin thin filaments in ordered arrays) is also present at the termini of microfilament bundles in non-muscle cells. In keeping

with this are Geiger and S. J. Singer's findings (*Cell* **16**, 213; 1979) that various specific extracellular ligands cause receptors to accumulate into patches and caps on the surfaces of various cells at the same time that  $\alpha$ -actinin forms sub-caps at the cytoplasmic face of the membrane. Inferences to be drawn from this include the possibility that  $\alpha$ -actinin provides the hinge between actin and integral membrane receptors, or that  $\alpha$ -actinin may collect together actin-linked receptors. This takes on further shape for adherent fibroblasts in Badley *et al.*'s recent work (*Expl Cell Res.* **117**, 231; 1978) in which cell bodies were removed from glass by a stream of buffer, leaving 'feet' behind in the same pattern as they existed in the intact cell. All of the components discussed so far in terms of a trans-membrane assembly could be detected by immunofluorescence at these adhesion plaques: actin, myosin, tropomyosin,  $\alpha$ -actinin, LETS (but not tubulin or serum albumin).

Putting all this together, fibronectin is a sticky protein which agglutinates red blood cells (Yamada *et al.* *Proc. natn. Acad. Sci. U.S.A.* **72**, 3158; 1975); has a binding site for collagen (Hahn and Yamada, *Proc. natn. Acad. Sci. U.S.A.* **76**, 1160, 1979); may be closely associated with sulphated proteoglycans (Perkins *et al.* *Cell* **16**, 941; 1979) and may therefore help organise biological matrices. It is concentrated at points of cell contact and can be traced

across the membrane to actin microfilament. Just as the external contact may be stabilised by components of the extracellular matrix, so the organisation at the internal face of the junction may be underpinned by  $\alpha$ -actinin. However, any communication across the membrane is likely to be two-way because external fibronectin stimulates internal microfilament bundle formation while, conversely, breakdown of the bundles by cytochalasin B (Kurkinen *et al.* *Expl Cell Res.* **111**, 127; 1978) causes the release of fibronectin from the cell surface.

Of course, there is strong evidence that the cytoskeleton can be modulated indirectly by the addition of cyclic nucleotides to cells (see Pastan and Willingham *Nature* **274**, 645; 1978) but in cases where the contractile cell flexes its muscle against the outside world (in attaching to cells and other biological surfaces, during cell migration and phagocytosis), a direct linkage is likely to be of prime importance. It has been suggested that many classes of external peripheral proteins cluster against one common class of trans-membrane protein which alone holds the hot-line to cytoplasmic microfilaments (Bourguignon and Singer, *S. J. Proc. natn. Acad. Sci. U.S.A.* **74**, 5031; 1977). The name given to this postulated molecule is perhaps an eloquent summary on the current debate about how outside and inside hinge together—it has been called 'protein X'. □

## Nucleic acid statics and dynamics

from Stephen Neidle

RECENT years have seen a revival of interest in structural studies of both nucleic acids themselves and of model systems for them. The impetus for this can be attributed to various causes—it comes from work on chromatin and transfer RNA; from visualisation of the structures of nucleic acid fragments, sometimes complexed with drug and mutagen molecules, and from the emergence of new and powerful techniques, especially the advent of high-resolution nuclear magnetic resonance spectroscopy. It was perhaps timely that at a recent meeting on 'molecular stereodynamics'\* half the sessions were concerned with such topics.

Even though hypothetical non-double-helical models for DNA are largely discounted (Arnott *Nature*

**278**, 780; 1979; Crick *et al.* *J. molec. Biol.* **129**, 449; 1979), there is lively interest in non-classical double helices. Levitt's analysis from empirical energy function calculations has suggested that in solution DNA has about 10.5 rather than 10 base pairs per turn, with the base normals markedly tilted from the helix axis and the base pairs themselves having propeller-like twists (*Proc. natn. Acad. Sci. U.S.A.* **75**, 640; 1978) D. M. Crothers (Yale) presented data from transient electric dichroism studies on drug-DNA intercalation complexes which seem to support some features of the Levitt model. In these structures, the drug chromophores are tilted by an average of about 20° from the perpendicular to the helix axis; nonetheless the nucleic acid was not observed to bend or kink *à la* Sobell (*J. molec. Biol.* **114**, 333; 1977). Such observations can only be rationalised by evoking partial unstacking and tilting of base pairs at the intercalation site. This in turn suggests a base-pair twist angle in DNA of 17°,

as does Levitt's model. L. S. Lerman (State University of New York, Albany), the originator of the intercalation concept, reported on his studies of DNA torsional elasticity probed with spin-labelled intercalators. Evidence was presented for substantial internal movement within the double helix; a single base pair oscillates about the helix axis with a root mean square amplitude of about 5°.

Nevertheless detailed structural data on nucleic acid-drug intercalation complexes (as opposed to hypothetical models), is still largely lacking. A favoured indirect approach to this problem is through the study of model systems. The pioneering crystallographic studies of H. M. Sobell (University of Rochester) on drug-ribodinucleoside complexes have clearly illustrated the intercalation phenomenon at this level. Sobell interprets these structures in terms of DNA complexes possessing kinks at the intercalation sites—this in turn has led him to suggest that DNA is a

\*A conversation on Stereodynamics of Molecular Systems was held at the State University of New York, Albany, on 23-24 April, and was organised by R. H. Sarma under the sponsorship of the State University of New York and the General Electric Company.

dynamically kinked-unkinked structure. The basis for kinking, alternating sugar pucker of residues at the intercalation site, was challenged by the results of H. M. Berman (Institute for Cancer Research, Philadelphia) and S. Neidle (King's College London), who find that for dinucleoside complexes, the nature of the sugar pucker (a parameter known to be 'soft') is probably unimportant. A. Rich (Massachusetts Institute of Technology) has also examined the crystal structures of several of these model intercalation complexes, and concludes that the expression of sugar flexibility in them depends on whether or not the particular drug involved interacts with the phosphate group in the backbone. It is not clear to what extent such drug-ribodinucleoside complexes are meaningful models for the drug-DNA complexes themselves. As Berman emphasised, great caution is needed in extrapolating from the model systems. Indeed, the data presented by Crothers suggest that the model complexes may be related to the DNA ones in rather more subtle ways than hitherto believed.

High-resolution nuclear magnetic resonance (NMR) is in principle a powerful probe of oligonucleotide conformation in solution, so as to provide data complementary to that from the crystal structures, as well as dynamic information. However, complete analyses of the spectra in terms of molecular geometry of even a relatively simple system such as a drug: dinucleoside one, have not been made. In spite of this proviso, NMR has been most useful in the study of many aspects of intercalation complexes. T. R. Krugh (University of Rochester) has shown that the simple intercalators such as ethidium show a marked sequence preference on binding, with pyrimidine-purine ones being preferred. This is the reverse of the preference shown by actinomycin D, which Krugh and his associates have shown to exhibit sometimes unexpected nucleic acid-binding behaviour. For example, although it has been known for many years that the drug requires a guanine at its binding site (and thus does not bind to poly(dA-dT).poly(dA-dT)), the anti-tumour intercalator daunomycin, which does bind to this polymer, actually creates noncompetitive actinomycin binding sites.

D. J. Patel (Bell Laboratories) has been examining by proton NMR the structural dynamics of synthetic polynucleotides both with and without intercalating drug. He presented further evidence for the 'softness' of

the sugar pucker in such systems. However, it is not clear whether, at least for an intercalative complex, the mixture of sugar conformers observed indeed represents a true alternating sugar situation (as propounded by Sobell), or a mixture of conformational populations. The power of the NMR method in conjunction with molecular model-building was well shown by R. H. Sarma (State University of New York, Albany) and his colleagues, who have examined models for the novel vertically stacked double helix suggested by Olson (*Proc. natn. Acad. Sci. U.S.A.* **74**, 1775; 1977). The concept of nucleic acid flexibility was further taken up by N. R. Kallenbach (University of Pennsylvania), who has examined the equilibrium and dynamics of transient base-pair breakage by following proton exchange rates, a simple yet powerful structural probe. Kallenbach concludes that indeed there are transient open states (analogous to Sobell's picture of DNA 'breathing').

Gleaning of dynamic properties from crystallographic results is necessarily an indirect process. However, both Rich and S.-H. Kim (University of California, Berkeley) valiantly attempted to confront this problem armed with their data from both small oligonucleotide and transfer RNA studies. Nucleic acids are inherently flexible, and tend to respond to their environments (be they drugs, counter-ions or proteins), in well defined, or at least preferred ways. It is apparent that even short stretches of double helix can easily be bent under the influence of directional ionic forces; in transfer RNA the axis of one helical stem has been observed to bend by some 25°. More controversially, Rich suggests that DNA winding around the histone core in chromatin is due to histone location on only one side of the double helix. The related problems of solvent accessibility and exposed surface area in nucleic acids were discussed by Kim, who emphasised the importance of knowing which are in fact the penetrable regions of these molecules, when considering possible interactions. By considering the Van der Waals radii of the atoms in a nucleic acid, he has shown, for example, that in (classical) B-DNA the minor groove can accommodate only very small probes. This distinctive method of looking at nucleic acids gives essentially a static picture; however Kim considers that the use of individual atomic thermal parameters as a description of motion may well lead to knowledge of dynamically accessible surface areas.

There are perhaps more distinct theoretical approaches to the prediction of nucleic acid conformation



## A hundred years ago

THE Italian State Secretary for Public Buildings has sanctioned the plans submitted to him for the construction of an observatory on the summit of Mount Etna.

THE Anthropological Exhibition at Moscow seems to be one of great interest. It is contained in a vast building lent by the Minister of War, and is used in winter for drilling soldiers, and the exhibition has been rendered as picturesque as it is scientific. A garden which has been arranged most artistically for the purpose presents, among other features, a very remarkable "palæontological valley." This is planted with lycopods, gigantic ferns, and other fossil plants; this forest is inhabited by models representing megatheriums, mammoths, ichthyosaurs, &c. On miniature mountains, the age of which is indicated by artificial geological sections, are shown *fac-similes* of Russian, French, Danish, and other tumuli. Besides this, an ethnological garden is peopled with models representing the principal human types, especially those of Russia. There is, besides, a remarkable anatomical and craniological exhibition. Altogether this is one of the most remarkable anthropological exhibitions ever brought together, and has been an immense success.

DR MICLUCHO MACLAY the Russian explorer, with an Italian, Chevalier Bruno, and Capt. Leeman, have sailed from Sydney for New Guinea, in the American schooner *Laddie*, F. Caller, chartered for a twelvemonth's cruise. 2,500*l.* has been spent on the equipment. The expedition is intended to be both scientific and commercial. New Caledonia, New Britain, and other islands are to be visited.

From *Nature* **20**, 5 June, 131, 134; 1879.

than there are actual practitioners of such arts. The experimentalist tends to treat all of them with a healthy scepticism. It was thus gratifying that S. Broyde (New York University), reporting on her semi-empirical energy calculations with B. Hingerty (Oak Ridge National Laboratory), with a model for the coil form of the poly(U) conformation, was able to show that the low-energy form suggested agrees with hydrodynamic and NMR data on poly(U). W. K. Olson (Rutgers Univer-

sity) has used the methods of polymer chain statistics in assessments of DNA flexibility dependence on chain length. Her results suggest that very small changes in just a few conformational parameters of individual residues lead naturally to very considerable flexibility in the polymer as a whole.

These contributions signify some of the more promising directions in which nucleic acid structural studies are going. All of these, however 'biological' the nucleic acid environment may be in an experimental situation can only provide *in vitro* data. One must look in the future to techniques such as those involving NMR described by S. S. Danyluk (Argonne National Laboratory), for information from intact biological systems. □

## Scrambling of Rydberg states by thermal radiation

from Peter Knight

ALL atomic systems are immersed in a bath of blackbody radiation, a background thermal field characterised by the ambient temperature of the body. For the most part physicists interested in radiative transitions of excited atoms just ignore the existence of this field, principally because the number of blackbody photons with a frequency close to the atomic transition under study is vanishingly small, so that stimulated processes due to background thermal fields are negligible. Nevertheless, if the transition frequency is small enough, the Planck distribution of thermal photon numbers ensures that stimulated emission and absorption will begin to take over from purely spontaneous radiative decay.

Recent experiments by Gallagher and co-workers at the Stanford Research Institute have indicated that transitions between very highly excited Rydberg states are sensitive to background thermal fields. At the transition frequencies that interest them, the thermal photon occupation number at room temperature can be as high as 10. The stimulated absorption and emission of this background radiation rapidly redistributes an initially excited state population among its neighbours and considerably shortens the lifetime of some states. Consequently it would seem quite difficult to observe the decay of a

single isolated Rydberg state in these circumstances: after a very short time a distribution of states rather than a single state exists.

In their first paper, Gallagher and Cooke (*Phys. Rev. Lett.* **42**, 835; 1979) report the observation of the shortening of the sodium radiative *np* lifetimes by a factor of 3 by 300 K blackbody radiation induced emission and absorption. They measured the effective lifetime of the 17p and 18p states of sodium produced by sequential excitation by two dye laser pulses driving the  $3s \rightarrow 3p \rightarrow 18p$  or 17p transitions. These states can be detected by field ionisation by a d.c. field, with a pulsed technique which allows them to monitor the time-evolution of the excited state population. Their results indicate that the lifetimes are a factor of three shorter than the calculated  $T=0$  K lifetimes, in agreement with their fairly simple analysis. They believe discrepancies between theory and experiment for transitions observed by other workers can be explained by thermal background effects. They also calculate that radiative redistribution of population among neighbouring states of different *l*-quantum number due to the thermal field can be as important as collisional redistribution. This could well have astrophysical implications. Gallagher and Cooke also calculate the a.c. Stark frequency shifts produced by the thermal field. These had, in fact, been investigated theoretically some years ago by Barton (*Phys. Rev.* **A5**, 468; 1972) and by Knight (*J. Phys.* **A5**, 417; 1972), and the results of Gallagher and Cooke are in agreement with those earlier quantum electrodynamic calculations. The thermal field shifts all the close-lying Rydberg states upwards in energy by an equal amount of the order of kilohertz. Low-lying states are shifted by a much smaller amount according to the theoretical work of Barton and Knight. So photoabsorption from a low-lying state to a Rydberg state may well exhibit temperature-dependent shifts, although these have yet to be seen. In a further paper, Gallagher and Cooke discuss the use of Rydberg atoms as a detector of 300 K blackbody radiation (*Appl. Phys. Lett.* **34**, 369; 1979), hinging on the sensitivity of field-ionisation as a monitor of highly excited state populations. They suggest that temperatures of less than 25 K could be detected by Rydberg-atom systems.

It is interesting to the theorist that the atom immersed in a radiation field is quite different from an isolated atom. The radiation bathing the atom also dresses it, so that what started as an isolated state is scrambled into a distribution of nearby states with transition frequencies determined at least in

part by the local environment. Hot atoms aren't just moving faster than cold atoms (and therefore the ensemble emitting a wider Doppler-broadened line): each hot atom is distinctly different from a cold atom in its radiative properties. □

## Proteins of iron metabolism

from Pauline Harrison and E. R. Huehns

THE main topics discussed at the recent, fourth International Conference on Proteins of Iron Metabolism\*—transferrin, the iron transport protein, and ferritin, the iron storage protein—are both in their own way still subject to considerable controversy. Transferrin has long been known to have two iron-binding sites, but whether these are identical structurally and, if different whether this has physiological importance, as Fletcher and Huehns proposed (*Nature* **218**, 1211; 1968), has been argued about at the previous three meetings.

B. Gorinsky (Birkbeck College, London) presented a '6Å resolution model' of rabbit transferrin while J. E. Abola from Pittsburgh had studied hen ootransferrin. The rabbit molecule consists of two lobes which can be described approximately by ellipsoids. The longest axes of these ellipsoids are inclined at about 30° to one another. The maximum overall dimensions of the molecule are approximately 100×50×40 Å. Both lobes exhibit clefts in the vicinity of the join. In one lobe the cleft is close to a column of strong electron density which could be  $\alpha$  helix, and on the opposite side of this lobe there is a strong feature which at higher resolution may prove to be  $\beta$  sheet. From other work it can be concluded that there is one iron-binding site on each domain and that the N-terminal binding site can be distinguished from the C-terminal one. Several investigators (J. Williams, Bristol; P. Aisen, New York; H. G. Van Eijk, Rotterdam) utilised electrophoresis in 6 M urea, as described by Seal and Mackay (*Biochim. biophys. Acta* **453**, 250; 1976), to separate the four species of transferrin—apotransferrin, diferric transferrin, and the two species of monoferric transferrin, one with iron on the N-terminal site only and the other with iron on the C-terminal site only—and to measure their distribution after the addition of iron to apotransferrin *in vitro* and in

\*Held in Davos on 17–21 April 1979.

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plasma. The results show that the distribution of iron on the two binding sites is not random both in *in vitro* loading experiments and in plasma *in vivo*. How far the results will confirm the detailed predictions of the so-called Fletcher-Huehns hypothesis has not been determined. But the non-equivalence of the two iron-binding sites and the non-random distribution of iron on them in plasma seems to be established and is presumably of some physiological importance.

An interesting approach to the evolutionary significance of transferrin was that of F. M. van Bockxmeer and E. H. Morgan (Western Australia) who compared the binding of transferrin to heterologous receptor cells and, from the relative affinities, constructed an evolutionary family tree for transferrin. Several investigators attempted to isolate the transferrin receptors from reticulocytes (J. Glass, Boston) or the placenta (E. B. Brown, Hong Kong and St Louis), but this work is still in its early stages.

Since the conference in 1977, advances have been made in the determination of the structure of the iron-storage protein, ferritin. P. Harrison (University of Sheffield, UK) described the interpretation of the 2.8 Å resolution electron density map of horse spleen apoferritin, in terms of a symmetrical arrangement of 24 equal subunits of 60%  $\alpha$ -helix content. The sequence determination is nearly complete (R. R. Crichton, Louvain-la-Neuve, Belgium) and interpretation of the three-dimensional map with sequence data is in progress. A complete description of this structure, now in sight, opens up the prospect of being able to settle arguments on how the ferritin molecule accumulates and releases its iron. Perhaps it may also lead to eventual agreement on the problem of ferritin heterogeneity, still a confused area. Differences in ferritin molecules both within and between preparations have been observed by electrophoretic and immunological methods. It now seems clear that these differences cannot all be explained by a single unifying hypothesis, such as that of J. Drysdale (Boston) who has proposed that all ferritins are heteropolymers of two subunits of different primary structure. Observations of apparent subunit size differences persist in some ferritins, although some types of reported heterogeneity may be methodological artefact. It now seems

unequivocal that ferritins from different cell types may differ in charge. For example, this was reported for ferritins from rat liver Kupffer cells and hepatocytes by J. W. Halliday (Brisbane, Australia) and from bullfrog red cells and liver cells by E. C. Theil (Raleigh, N. Carolina) who also reported amino acid composition differences.

Several workers produced evidence that iron-loading changes the 'isoferritin' pattern, but it is not clear whether this results from selective synthesis and/or degradation, or from post-translational modification. M. Worwood (Cardiff, UK) found that a fraction of serum ferritin is modified by glycosylation, a fraction which may alter in diseased states. These workers also reported that the immunological properties of circulating ferritin in patients with malignant disease are similar to those of ferritin from normal spleen. A molecule with high iron-content (significantly different from ferritin) has been found in *E. coli* grown on iron-rich media by E. R. Bauminger (Jerusalem and Rehovoth, Israel), suggesting that a means of conserving iron or of compartmentalising unneeded iron, is more widely required than hitherto recognised. On the other hand, iron-storage may not be the whole story with ferritin. Immunosuppression in patients with Hodgkin's disease may be partly linked with the presence of ferritin on their lymphocyte surfaces.

The last session of the conference concerned the management of transfusional iron overload, and although there were no new approaches, several investigators confirmed that maximal iron excretion could be best achieved using daily subcutaneous desferrioxamine with vitamin C supplements. The production of animal models of iron overload was also described by G. McLaren (Cleveland) and M. Awai (Okayama, Japan). □

## Ion-driven inertial fusion

from Richard C. Arnold

THE first experiments using pulses of light ions to implode a hollow target, which might in the future be scaled up to produce inertial-confinement fusion, have been reported (*Phys. Rev. Lett.* **42**, 610; 1979) by a group at the

Sandia Laboratories in the US.

Previous experiments have been carried out for some years with lasers and electron beams (see Stickley *Phys. Today* **50**; April 1978); thermonuclear burn has been achieved in both cases. The light ion driver experiments reported, with pulse energies of 0.5 terawatt, are as yet at least one order of magnitude too weak to produce a measurable neutron yield (in fact, no attempt was made to use deuterium or tritium), but are sufficiently powerful to allow meaningful diagnostics of target response.

Ion beams can be produced with high electrical efficiency, and deposit their energy very effectively in the metallic surface of a fusion target. In these respects light or heavy ion-beam drivers are superior in principle (compare *Sci. Am.* **239**, 50; 1978) to existing laser systems. However, the physics and technology required to arrange focusing of intense ion beams on a small target for implosion purposes has been questioned, particularly for light ions of low kinetic energy ( $\sim 1$  MV) and high currents ( $\geq 1$  MA) required for an inertial-fusion reactor. The Sandia experiments have demonstrated that a major fraction of their ions can be deposited on thin ( $\sim 4 \mu\text{m}$ ) conical target shells about 1 cm in diameter, driving the shell inward in an axially symmetrical implosion. A plasma temperature of 12 eV was achieved at the peak of collapse (thermonuclear burn in this configuration would probably require about two orders of magnitude greater temperature).

The pulsed-power technology used in these light-ion experiments is relatively inexpensive compared with the heavy-ion multi-gigavolt accelerator technology (see *Nature* **276**, 19; 1978) now under development with a view to commercial power applications. As a consequence, fusion target experiments with light ions will probably be done much earlier than in the US heavy-ion programme. The latter technology, however, has many potential advantages for commercial reactor design. For example, heavy-ion beam propagation over several meters in a reactor vessel is expected to be classical and relatively straightforward; while for light ions, such long-distance propagation must be carefully arranged through a pre-ionised neutralising plasma channel, raising serious questions of possible propagation instabilities at the low kinetic energies used. Propagation and focusing on a small spot ( $\ll 1$  cm) thus need to be demonstrated with light ions, and several groups in the US (at Sandia, Cornell University and the Naval Research Laboratory) are investigating this question. □

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## A decade of plate tectonics

from A. Hallam

J. TUZO WILSON is widely acknowledged as one of the founding fathers of the new global tectonics. Because he has been successively Professor of Geophysics and Principal of Erindale College in the University of Toronto, before taking up his present position of Director of the Ontario Science Centre, it is appropriate that the university recently held a conference\* to honour his multifarious contributions to earth science. Although various topics were discussed, including the early thermal history of the Earth, electromagnetic studies of the crust and the origin of ore deposits, the dominant theme was plate tectonics. Approximately a decade has passed since plate tectonics became generally accepted, and the meeting provided a valuable means of finding out how the subject was now being studied in a range of disciplines and of taking stock of the current state of knowledge.

Much of the critical research leading to the earth sciences revolution was in the fields of rock magnetism and seismology, and several leading workers were present to outline recent advances. Both A. Cox (Stanford University) and E. Irving (Earth Physics Branch, Energy, Mines and Resources, Ottawa) indicated how detailed palaeomagnetic studies in alliance with geological research demonstrate that extensive sectors of the Western Cordillera of North America had either rotated or been translated northward for substantial distances in the late Mesozoic and early Tertiary. While Irving was concerned essentially with northward movement of two landmasses, Wrangellia and Stikina, Cox dealt mainly with inferred rotations. To apply sailing terms, the structural geologist studies pitch and role but only the palaeomagnetist can determine the yaw. D. York (University of Toronto) showed how a combination of palaeomagnetic and isotope studies could help to unravel complex events in the Precambrian and confirm, for example, the reality of the polar wandering event about  $10^9$  years ago known as the Grenville Loop.

A quite different approach to rock magnetism was adopted by F. J. Vine (University of East Anglia) who concerned himself with the record of reversals of the geomagnetic field revealed by ocean floor anomalies, and demonstrated how further detailed re-

search could reveal information on variations in intensity of the field in space and time, which can delimit changes in the strength of the dipole and non-dipole field components with time.

Seismological data originally provided strong evidence for the delimitation and character of plate boundaries, and attention is now being directed more to seismicity within plates. L. R. Sykes (Lamont-Doherty Geological Observatory, New York) indicated that intraplate earthquakes tend to be concentrated along pre-existing zones of weakness affected by the youngest orogeny that predates the opening of the present oceans. Many such zones, marked by fault and suture lines and failed rifts, were reactivated during the early stages of continental separation. In contrast, intraplate shocks rarely occur within the older oceanic lithosphere or within ancient continental cratons.

The seismic reflection profiling method, developed by the oil industry for the study of sedimentary basins, is now being applied to the study of the structure of the continental basement. The most interesting result so far, as described by J. Oliver (Cornell University) is the discovery that the Blue Ridge and Piedmont provinces of the Appalachians are allochthonous and have been thrust at least 275 km westwards.

Appropriately enough, considerable attention was paid to the Wilson Cycle of ocean opening and closure, the principal preoccupation being the character of subduction zones. S. Uyeda (Earthquake Research Institute, Tokyo) presented a model of two contrasted types of subduction zone exemplified by the eastern and western sides of the Pacific, which was developed in more detail by J. F. Dewey (State University of New York, Albany). Flat subduction trajectories produced by flat mantle flow and/or the subduction of young lithosphere produces pervasive compressional strain in the overriding slab at high slip-rates. Steep subduction trajectories, subduction of old lithosphere and low slip-rates induce back-arc spreading. Dewey went on to argue that plate tectonics in its present form has been in operation for about  $2 \times 10^9$  yr, a view supported by P. Hoffman (Geological Survey of Canada) in his description of an early Proterozoic Wilson cycle in northern Canada.

Similarly, there was accord between Dewey and W. R. Dickinson (Stanford University) that a tectonic mosaic involving horizontal motions was already in existence in Archaean times, and the model of early continental evolution put forward by S. Moorbath (Uni-

versity of Oxford) was consistent with this interpretation. Moorbath argued strongly that isotopic data pointed to continental growth by irreversible differentiation of the upper mantle throughout geological time, rather than by reworking and regeneration of much older crust. Intriguingly enough, Tuzo Wilson was once most widely known for his notion of continental accretion, albeit at a time when he was a staunch opponent of continental drift.

Other aspects of subduction were dealt with by W. S. Fyfe (University of Western Ontario), who argued for substantial subduction of sediments, a view contested by Moorbath, and A. Miyashiro (State University of New York, Albany). Miyashiro concerned himself with metamorphism and estimated that W. G. Ernst's successive models of the high pressure—low temperature zone had greatly improved our detailed understanding, whereas serious problems remained with the low pressure—high temperature zone, notably the origin of the magmas generated there. It seems that the old, widely accepted model assuming magma generation by simple partial melting of the oceanic crust in a descending slab is no longer tenable.

A reassessment of Panagaea reconstructions and time of Mesozoic breakup was undertaken by A. Hallam (University of Birmingham). The familiar 1-km isobath computer reconstructions were inaccurate in detail because it is now known for many regions that the boundary of continental and oceanic crust must occur beneath 2–4 km or even greater depths of ocean, breakup having been preceded by extensive stretching and subsidence. Furthermore, there may be a long time interval between initial taphrogenic activity ('rifting') and creation of ocean floor by spreading ('drifting'). A. J. Boucot (Oregon State University) expressed scepticism about reconstructions of changing continental positions in the Palaeozoic and attempted to show by plotting fossil and climatically significant sediment distributions that an unchanging Panagaea was as satisfactory a model as any. On the other hand, H. Williams (Memorial University, Newfoundland) argued that geological evidence in the Appalachians supported the view that the Iapetus was a major ocean. Problems indeed remain in reconstructing continental positions in the Mesozoic and Cainozoic, but they seem to be child's play in comparison to earlier periods of time. Our best hope would seem to rest with the palaeomagnetists. □

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\* The symposium was held at the University of Toronto on 14–16 May 1979 and the proceedings will be published as a special paper of the Geological Association of Canada.

# review article

## Quantum chromodynamics

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*There has been a growing conviction during the past few years within the high-energy physics community that a fundamental theory of the strong interaction has been found. This theory is called quantum chromodynamics (QCD) and if correct it will be a major triumph for theoretical physics. In this review we trace the experimental and theoretical developments which gave rise to QCD, outline its basic properties and discuss its successes and remaining problems.*

THE fundamental forces in nature are conventionally divided into four categories. They are, in order of their apparent strengths, strong, electromagnetic, weak and gravitational interactions. Two of these, electromagnetic and gravitational interactions, are long range and hence dominate our physical surroundings; so they are the most familiar and best understood. On the other hand, both strong and weak interactions are extremely short range (they only occur over distances  $\leq 10^{-13}$  cm), so their relevance and study are primarily limited to the disciplines of nuclear and elementary particle physics. The weak interaction is responsible for phenomena such as  $\beta$  decay of neutrons into protons and neutrino interactions with matter; in addition it violates some otherwise exact symmetries of nature such as parity and time reversal invariance. The weak interaction gained notoriety recently because of the success of the Weinberg-Salam model<sup>1,2</sup> in combining weak and electromagnetic interactions into one theory. Finally, there are the strong interactions, which had until recently been poorly understood; but some very significant advances have been made and these are reviewed here.

The strong interaction is primarily known for its role as the powerful short-range force that binds protons and neutrons together inside atomic nuclei. In that capacity, the nuclear binding force is usually described as being due to the exchange of mesons (strongly interacting integer spin particles) such as pions between nucleons. High-energy accelerator experiments during the past several decades have shown that nuclear binding is merely one of many manifestations of the strong interaction. We now know that, in addition to the proton, neutron and light mesons, many other hadrons exist which are produced and interact with one another via the strong force. (The term hadron refers to any strongly interacting particle.) There are evidently an infinite number of hadrons which vary from the familiar stable proton to highly unstable resonances which live for much less than a billionth of a second in the laboratory. The task of a fundamental theory of the strong interaction is to account for all these diverse hadrons and to provide a dynamical description of their properties.

The current view is that the observed hadrons are not elementary particles but are bound states of truly elementary constituents called quarks, and that strong interactions between hadrons are merely epiphenomena of the more fundamental forces between quarks. Of course, the forces that bind quarks together inside hadrons must be extraordinarily strong, as experiments have not revealed an isolated free quark. Furthermore, we now believe that the strong interactions between quarks may be fully described by a quantum field theory called quantum chromodynamics (QCD). We describe here some of the major experimental and theoretical developments which gave rise to the emergence of QCD as a viable theory of the strong interaction.

### The quark model

THERE are hundreds of known hadrons (an infinite number if excitation levels are included) which exhibit widely diverse properties. This observed spectrum of hadrons is divided into two classes, mesons which have integer spin (0, 1, 2 ...) such as the pion ( $\pi$ ), kaon (K), and rho ( $\rho$ ) and baryons which have half-integer spin (1/2, 3/2 ...) such as the proton (p), neutron (n), lambda ( $\Lambda$ ), and omega ( $\Omega$ ). Examination of their specific properties indicates that mesons and baryons can be further subdivided or grouped into multiplets in which the members are all quite similar. This classification scheme is called the eightfold way<sup>3</sup>; it provides the same kind of organisation for hadrons as the Periodic Table does for chemical elements. It was the regularity of these multiplet groupings that led Gell-Mann and Zweig to conjecture that all hadrons could be built up as bound states of a few fundamental spin 1/2 constituents called quarks. Their conjecture is the basis of the quark model<sup>4-7</sup>. In this scheme, mesons are bound states of a quark (q) and antiquark ( $\bar{q}$ ), while baryons are constructed from three quarks qqq (antibaryons are made out of three antiquarks  $\bar{q}\bar{q}\bar{q}$ ). (Note that an antiparticle has the same mass as a particle but exactly opposite quantum numbers.) The mesons and baryons so constructed should have integer electric charge, even though their

constituent quarks carry fractional charge. Using these simple rules one can build all the observed hadrons out of quarks. The quark model has been very successful in predicting new hadronic states and correctly describing their properties. It is one of the cornerstones of elementary particle physics.

Five distinct species of quarks, or flavours, are known. In order of increasing mass, the quark flavours are up (u), down (d), strange (s), charm (c) and bottom (b). It is also generally believed that a sixth flavour, the top quark (t), exists at an energy somewhat higher than that now probed by accelerators. The up and down quarks are the lightest; next comes the strange quark which is about 50 times heavier than the up quark. All hadrons discovered before 1974 (the year of the  $J/\psi$  particle's discovery<sup>8,9</sup>) are built up out of these three flavours (u, d, and s); that is why they formed the basis for the eightfold-way classification scheme. Charm and bottom flavours are more recent additions to the quark model. They are respectively about 375 and 1,125 times heavier than the up quark, so their existence could only be uncovered by modern day high-energy accelerators. The top quark's mass is thought to be larger still; there may also be other even heavier flavours waiting to be discovered. All quarks carry fractional electric charge; the d, s and b have charge  $-1/3$  (in units of the proton's charge) while the u, c and t (?) have charge  $+2/3$ . These, with other quark properties, are shown in Table 1.

**Table 1** Quark flavours and their properties

Quark flavour	Symbol	Mass ratios*	Charge	Baryon no.	Spin
Up	u	1	$+2/3$	$1/3$	$1/2$
Down	d	2.5	$-1/3$	$1/3$	$1/2$
Strange	s	50	$-1/3$	$1/3$	$1/2$
Charm	c	375	$+2/3$	$1/3$	$1/2$
Bottom	b	1,125	$-1/3$	$1/3$	$1/2$
Top (?)	t	3,375 (?)	$+2/3$	$1/3$	$1/2$

\* With respect to  $m_u$ ; it is usually estimated that  $m_u \sim 4$  MeV.

Within the quark model framework, all known hadrons can be built up out of the five quark flavours u, d, s, c and b (see Fig. 1). For example, the proton is a bound state of one down and two up quarks while the neutron is made out of one up and two down quarks. Similarly, 'strange' hadrons can be made using the s quark. An example of a meson is the recently discovered spin 1  $J/\psi$  particle<sup>8,9</sup> which is now recognised as being merely one energy level of charmonium, that is a bound state of charm and anticharm ( $c + \bar{c}$ ). Similarly, an even more recent meson<sup>10</sup>, the upsilon  $T$ , is thought to be an energy level of bottomonium ( $b + \bar{b}$ ).

An assumed property of the strong interactions between quarks is that they are flavour independent and flavour conserving. This accounts then for the strong interaction conservation laws observed at the hadronic level such as isospin, strangeness, and charm conservation. Only weak interactions change the flavours of quarks. They are responsible for flavour changing decays of hadrons such as the beta decays  $n \rightarrow p^+ + e^- + \bar{\nu}_e$ , and  $\Lambda^0 \rightarrow p^+ + e^- + \bar{\nu}_e$ .

All our knowledge regarding quarks has been obtained indirectly from the properties of hadrons, as an isolated quark has never been observed. Given the outstanding success of the quark model, why have free quarks never been observed? Are quarks so tightly bound together that they cannot be dislodged from their hadronic domains? The apparent impossibility of liberating quarks has been one of the great puzzles of the quark model.

### Deep-inelastic scattering experiments

Although the quark model was very successful in classifying and accounting for the observed hadrons, there was no compelling

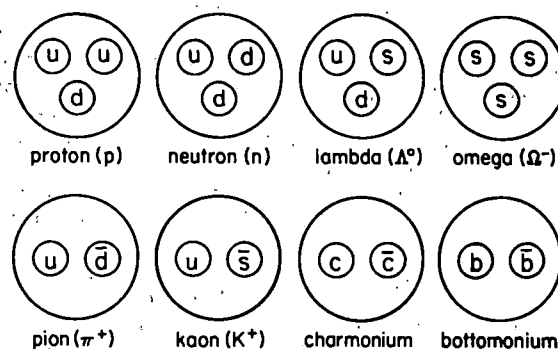
evidence that hadrons were actually built up out of quarks until the electroproduction experiments<sup>11</sup> were carried out at the Stanford Linear Accelerator Center (SLAC) in 1968. These experiments provided direct evidence that hadrons are really made up out of point-like elementary constituents.

The prototype SLAC experiment consisted of scattering a very high-energy ( $\approx 20$  GeV) beam of electrons on a proton target (see Fig. 2). (The electron (e) belongs to a class of elementary particles called leptons, which can interact electromagnetically and weakly but not strongly. Other known leptons are the muon ( $\mu$ ), tau ( $\tau$ ) and the associated neutrinos  $\nu_e$ ,  $\nu_\mu$  and  $\nu_\tau$ .) As electron-proton scattering proceeds via the electromagnetic interaction as shown by the virtual photon exchange in Fig. 2, it provides a good way of probing the proton's internal structure. If the proton's electric charge were diffused throughout the internal structure, then at very high energies the inelastic scattering cross-section would be expected to decrease very rapidly. That was not observed. Instead, the cross-section decreased slowly in a manner that suggested the proton's electric charge was concentrated in point-like constituents; these were the quarks inside the proton. When high-energy neutrino beams became available, they were also used as hadronic probes and predictions based on the quark model were again confirmed.

A surprising picture emerged from these deep-inelastic scattering experiments. Although the strong interactions of quarks seem to be very complicated at low energies, they become much simpler at high energies. Indeed, at very high energies (or equivalently at very short distances) it was found that quarks behave as though they were essentially free particles inside the hadron. This is called scaling behaviour<sup>12</sup>. Contrary to the behaviour of all other known fundamental forces, the strong interaction actually diminishes as one probes nearer the quark. An explanation of this remarkable dynamical property had to await the advent of QCD.

### Colour

In the late 1960s it became clear that the simple quark model was inadequate. In addition to flavour, quarks must carry an additional quantum number which has come to be known as colour<sup>13</sup>. Each quark flavour comes in three distinct colours; that is, there are really three times as many quarks as previously

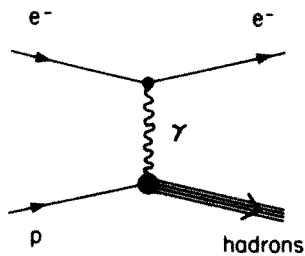


**Fig. 1** Pictorial description of the quark content of a few well known hadrons. Inside hadrons, quarks act like free point-like particles.

described. For example, there are red, green and blue up quarks denoted by  $u_r$ ,  $u_g$ ,  $u_b$ ; they are identical in all respects (mass, charge and so on) except colour. Similarly, the other flavours also come in these same three colours ( $d_r$ ,  $d_g$ ,  $d_b$ ,  $s_r$ ,  $s_g$ ,  $s_b$ , and so on). Why was this colour degree of freedom necessary?

Colour was originally introduced as a way of reconciling quark statistics with the observed low-lying baryon spectrum. As quarks have spin  $1/2$ , they should obey Fermi statistics, that is all quantum states should be anti-symmetric (change sign) under





**Fig. 2** Deep-inelastic electron-proton scattering proceeding through the electromagnetic interaction. The final state contains many hadrons.

the interchange of two identical quarks. However, this expectation seemed to be violated by the lowest energy baryons. For example, the  $\Omega^-$  (see Fig. 2) is made up of three spin 1/2 strange quarks; so according to Fermi statistics its wave function should be antisymmetric under the interchange of any two of these quarks. But the  $\Omega^-$  has total spin 3/2 so the spin part is symmetric; and as it is the lowest energy state of three s quarks, they ought to have zero orbital angular momentum relative to one another, thereby implying that the spatial part of the wave function is also symmetric. However, the total wave function is then symmetric under quark interchange in violation of Fermi statistics. This apparent inconsistency is overcome by giving each of the quarks an additional degree of freedom, colour. Then antisymmetrising the state in the quarks' colour variables renders the  $\Omega^-$  overall antisymmetric and resolves the problem. The antisymmetrised  $\Omega^-$  state is denoted by

$$(s_r^1 s_g^2 s_b^3 - s_r^1 s_b^2 s_g^3 + s_b^1 s_r^2 s_g^3 - s_b^1 s_g^2 s_r^3 + s_g^1 s_b^2 s_r^3 - s_g^1 s_r^2 s_b^3) 6^{-1/2}$$

where the superscript labels the three distinct quark wave functions; note that it changes sign if any two quarks are interchanged (for example,  $1 \leftrightarrow 2$ ), as required. In the same way all baryons are made antisymmetric in colour; they have zero net colour. (Our choice of the standard primary colours for the quarks was arbitrary; however, it helps convey the physics, as equal admixtures of these colours yield a colourless state.) Mesons are colourless because they are made from a quark + antiquark which must have opposite colour quantum numbers.

The introduction of colour also resolved other problems, one of which was the decay rate of the neutral pion into two photons,  $\pi^0 \rightarrow 2\gamma$ . The experimentally observed rate was nine times larger than the quark model's prediction. Introducing colour increased the number of intermediate quark states and the corresponding pion decay amplitude by a factor of 3. In this way the predicted rate was increased by the necessary factor of  $9 = 3^2$ , which is further convincing evidence for three colours.

Another indication of colour's requirement came from the quantity  $R$  which is the ratio of the cross-sections for electron-positron annihilation into hadrons and muons,  $R \equiv \sigma(e^+e^- \rightarrow \text{hadrons})/\sigma(e^+e^- \rightarrow \mu^+\mu^-)$ . Without colour, the experimental value of  $R$  was three times larger than the quark model's prediction. Introducing a factor of 3 for colour brings them into agreement. The three pieces of input, baryon statistics,  $\pi^0 \rightarrow 2\gamma$  decay rate and the value of  $R$  are the best available evidence for colour.

Why is this additional quantum number called colour? The nomenclature colour provides a convenient way of describing the fact that the observed hadrons do not carry this new quantum number, they must be colourless. It is equivalent to the mathematical statement that each quark flavour transforms as a triplet representation under an internal  $SU(3)_c$  colour symmetry; but physical hadron states are all singlets.

The quark model when appended with the  $SU(3)_c$  colour symmetry provides a good description of the observed hadronic spectrum. However, it fails to account for the dynamics of the strong interaction and leaves unanswered some obvious questions. Why do quarks inside hadrons behave as though they were free when we probe them in very high-energy experiments or

equivalently, why does the strong interaction's strength decrease in the vicinity of quarks? Why are free quarks or any other coloured hadronic states not observed in nature? QCD answers the first question and, we believe, may also answer the second one.

## Gauge theories

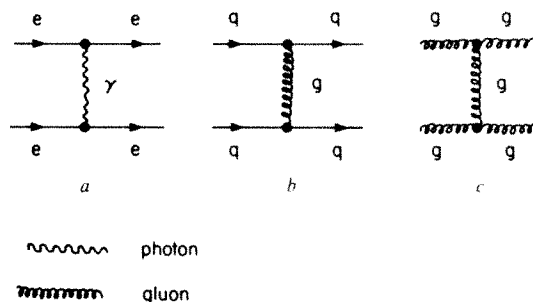
Quantum chromodynamics is a particular example of a general class of theories called non-abelian gauge theories. Significant developments in our understanding of these theories have occurred during the past decade which set the stage for QCD's emergence. Some of these advances will be outlined after we have briefly explained the fundamentals of gauge field theories.

The basic idea of a gauge theory is that a continuous symmetry or global invariance property of a lagrangian field theory can be made into a local invariance by introducing compensating gauge fields into the theory. This means that given a field theory which possesses a symmetry such as  $U(1)$ ,  $SU(2)$ ,  $SU(3)$  or any other Lie group, the theory can be extended to a gauge theory which has that symmetry at each point in space-time individually. The new symmetry is called a gauge symmetry because it implies that we can choose our measuring standard (gauge) (such as,  $SU(2)$  isospin basis) differently throughout space-time without changing the physics of the theory.

The most familiar example of a gauge theory is electromagnetism. In that case the symmetry group is  $U(1)$  corresponding to the freedom of changing the phase of any electrically charged field ( $\psi(x) \rightarrow e^{i\theta}\psi(x)$ ); a symmetry that follows from charge conservation. Extending this to a local  $U(1)$  gauge invariance ( $\psi(x) \rightarrow e^{i\theta(x)}\psi(x)$ ) requires the introduction of a single gauge field, the electromagnetic vector potential  $A_\mu(x)$ , which becomes the photon field in the quantum theory.

Quantum electrodynamics (QED), the quantum field theory of the electromagnetic interactions of charged particles and photons, is our most successful gauge theory. It is renormalisable, which means that all short distance (ultraviolet) divergences encountered in perturbation expansions can be absorbed into the definitions of the physical parameters (electric charge, masses, and so on); in terms of these quantities all predictions of the theory are finite. QED's predictions for precisely measured quantities such as the anomalous magnetic moments of the electron and muon and the Lamb shift in hydrogen are in perfect agreement with experiment. The incredible accuracy of QED strengthens our belief that renormalisable theories and gauge theories in particular, may also provide good descriptions of the other fundamental interactions.

The generalisation of the principle of local gauge invariance from the electromagnetic  $U(1)$  group to an arbitrary Lie group was accomplished by Yang and Mills<sup>14</sup> in 1954. Their work showed that the number of required compensating gauge fields equals the number of group generators and that in the case of



**Fig. 3** Diagrams depicting: a, electron-electron electromagnetic interaction via the exchange of a virtual photon; b, quark-quark strong interaction via the exchange of a virtual gluon; c, gluon-gluon strong interaction via the exchange of a virtual gluon. c illustrates the essential difference between QED and QCD (photons do not self-interact); this novel feature gives rise to asymptotic freedom and perhaps colour confinement as well.

non-abelian groups (SU(2), SU(3) and so on) the gauge fields interact among themselves; a novel feature with extraordinary consequences.

Non-abelian gauge theories lay dormant until two theoretical advances were made. The first was the development of a procedure for quantising non-abelian gauge theories by Faddeev and Popov<sup>15</sup>. It elevated them from classical field theories to quantum field theories in which the interacting fields could be identified with particles (quanta). The second was a formal proof of the renormalisability of non-abelian gauge theories which came from the work of 't Hooft and Lee and Zinn-Justin<sup>16,17</sup> in 1971–73. This put non-abelian gauge theories on as firm a theoretical footing as the successful QED, and made them potential candidates for describing the other fundamental interactions.

An immediate outgrowth of the work just described was renewed interest in the Weinberg–Salam model<sup>1,2</sup> of weak and electromagnetic interactions which is based on the gauge group SU(2) × U(1) (often called quantum flavourdynamics (QFD)). This theory describes and unifies all known weak and electromagnetic phenomena; it has been very successful in predicting now observed neutrino neutral current scattering cross-sections<sup>18</sup> and parity violating asymmetries in deep-inelastic polarised electron scattering<sup>19</sup>. These successes provide objective evidence that non-abelian gauge theories do describe physical phenomena.

## Asymptotic freedom

In 1973 a remarkable property of non-abelian gauge field theories was uncovered by Politzer and Gross and Wilczek<sup>20,21</sup>. They found that because of the self interactions among gauge fields, these theories exhibit the property of asymptotic freedom. That is, the strength of the interactions mediated by non-abelian gauge fields becomes vanishingly small at asymptotically high energies (or equivalently at very small distances). These theories behave almost like free field (non-interacting) theories in the asymptotic energy regime. This was precisely the property observed in the deep-inelastic scattering experiments: at high energies quarks inside hadrons behave as though they were free. Furthermore, it was shown that non-abelian gauge field theories are the only field theories which exhibit asymptotic freedom<sup>22</sup>. An obvious conclusion was that the strong interactions between quarks must be mediated by non-abelian gauge fields.

## Quantum chromodynamics emerges

The asymptotic freedom of non-abelian gauge theories fits beautifully with the quark model of hadrons and the requirement for three hidden quantum numbers called colour; from these distinct ideas and discoveries quantum chromodynamics (QCD) emerged (QCD is reviewed in ref. 23). The basic ingredients of QCD are the following: each quark flavour (u, d, s, c, b, and so on) comes in three colours (for example, red, green, and blue) which differentiate what would otherwise be three identical quarks. The three colours of quarks ( $u_r, u_g, u_b$ ) transform as a fundamental triplet of the group SU(3)<sub>c</sub> where the subscript c stands for colour. This SU(3)<sub>c</sub> symmetry is an exact symmetry of nature; it is never violated. The key new idea of QCD is that the SU(3)<sub>c</sub> symmetry must be a local gauge symmetry. This extension to a local SU(3)<sub>c</sub> gauge symmetry requires the introduction of eight vector gauge fields called coloured gluons (corresponding to the eight generators of the SU(3)<sub>c</sub> group) which transform as an SU(3)<sub>c</sub> octet. The gluons are massless spin 1 quanta which mediate the strong interaction between coloured quarks (see Fig. 3). In that way, gluons are to QCD what photons are to QED; however, an extremely important difference is that gluons carry colour and therefore interact strongly among themselves, while photons are electrically neutral and therefore cannot directly interact with one another. This distinction gives rise to asymptotic freedom in QCD (the effective coupling between

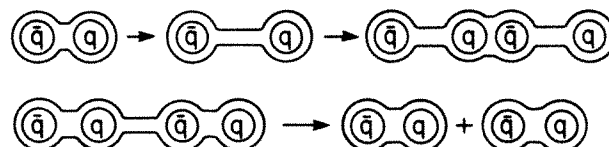
fields decreases at short distances) and perhaps other dramatic consequences as well.

The flavour quantum number of the quarks plays no role in the QCD strong interaction; all flavours of quarks interact strongly with one another in the same manner. Colour interactions are flavour blind while weak and electromagnetic interactions are colour blind.

The immediate success of QCD was that it incorporates all the earlier successes of the quark model with colour. Furthermore, this quantum field theory has all the observed strong interactions symmetries, flavour conservation, parity and time reversal invariance, and no additional unobserved symmetries. Also, if a theory of the weak and electromagnetic interactions such as the Weinberg–Salam model is appended to QCD it does not disturb these strong interactions selection rules except by small, calculable amounts, nor do the strong interactions disturb weak and electromagnetic phenomenology.

One can go beyond symmetries to real dynamics. The property of asymptotic freedom inherent to this exact non-abelian gauge theory accounts for the scaling phenomena observed in deep-inelastic electron and neutrino scattering experiments. It explains why at very high energies quarks act as though they were free particles inside hadrons. Furthermore, we now know that the scaling behaviour is not exact. There are small logarithmic deviations from exact scaling predicted by QCD and they are experimentally observed, a beautiful confirmation of the theory<sup>24</sup>.

The fact that QCD's strong interaction gauge coupling constant decreases as the energy domain probed increases (which follows from asymptotic freedom) allows one to calculate high-energy QCD effects perturbatively in this small coupling. Today an enormous effort is underway to compute QCD predictions for lepton–hadron and hadron–hadron scattering processes using perturbation theory. One observed effect that perturbative QCD accounts for nicely is the jet structure of final state hadrons produced in electron–positron annihilation ( $e^+e^- \rightarrow \text{hadrons}$ ) at high energies—the tendency of hadrons to be produced with very little transverse momentum in streams of



**Fig. 4** An unsuccessful attempt to free a quark from a bound state meson. The energy expended in pulling the quarks apart goes into creating a quark–antiquark pair out of the vacuum and thereby leads to two colourless mesons. Quarks and their colour remain confined.

narrow jet-like cones<sup>25</sup>. In principle it is possible to calculate QCD predictions perturbatively to arbitrary precision in such high energy kinematic regimes and compare with experiment; thereby rigorously testing the viability of QCD.

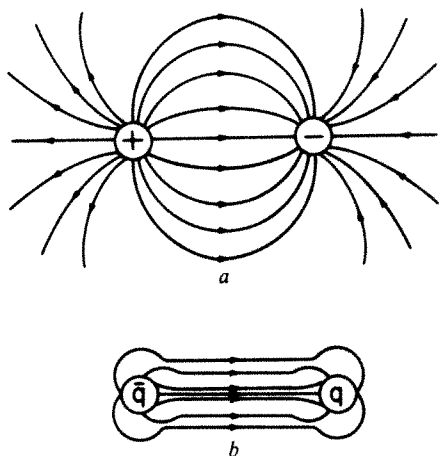
Another experimental-consistency check of QCD involves bound state mesons of heavy quark flavours. Heavy quark systems are a good test for QCD because the binding interaction is at short distances, so the coupling is diminished and perturbation theory becomes applicable. For example, charmonium which is a bound state of  $c$  and  $\bar{c}$  quarks has been vigorously investigated using QCD (charmonium is reviewed in ref. 26). In this way, the observed energy level splittings and various partial decay rates of charmonium have been reasonably well explained. The more recently discovered upsilon T, which is a bound state of  $b$  and  $\bar{b}$  quarks, should provide an even better system for investigating QCD effects as the coupling strength for that very massive quark system is even smaller.

Using QCD one should eventually be able to calculate hadron masses, the couplings between mesons and baryons, strong interaction cross-sections, essentially all strong interaction

phenomena. At present, the optimism surrounding QCD results from the fact that it is not contradicted where it can be checked experimentally. We can expect QCD to be subjected to more rigorous tests in the future.

There are still some outstanding problems confronting the QCD field theorist, the foremost being to prove the absolute confinement of colour. Because quarks, gluons and coloured bound states have never been observed, colour must be somehow confined (not physically observable) in QCD. It is believed at present that as one tries to break a colourless hadron apart into its coloured constituents, the potential energy grows linearly until there is enough energy to create new quark-antiquark pairs and gluons out of the vacuum. In this way the hadron can divide into several colourless hadrons, so that quarks, gluons and their colour remain confined (see Fig. 4).

One way that this scenario might be realised in QCD is through 'infrared slavery'—the opposite of asymptotic freedom. Infrared slavery means that the self-interaction of non-abelian gauge fields causes the inter-quark and -gluon couplings to become very large at large distances (perhaps infinite). This accounts for strong quark binding and might, hopefully, imply colour confinement.



**Fig. 5** *a*, Electric field lines between positive and negative electric charges. *b*, Sketch of the anticipated colour field lines between a quark and antiquark. The lines in (*a*) give rise to a Coulombic potential  $\sim 1/r$ , and those in (*b*) give rise to a linear confining potential  $\sim r$ .

Another possibility is that the colour gluon fields may form a flux tube or string between colour sources. If the coloured flux in the tube is conserved it is easy to show that the potential energy between sources rises linearly with the separation, implying confinement of colour (see Fig. 5).

Another idea is that the vacuum state surrounding a hadron is a very complicated strong coupling regime. The hadron is a little bag or bubble in this vacuum describing a two-phase system. The quarks and gluons inside are in relatively free motion. Only if the quarks or gluons try to escape from the bag do they experience strong confining forces. This is essentially the phenomenologic-

ally successful MIT bag model (see the review in ref. 27). The problem is to show that such a bag picture of hadrons actually follows from QCD.

There are other problems besides that of the colour confinement. The pion ( $\pi$ ) seems experimentally to be a collective excitation of quarks rather than an atomic bound state. This property of pions can be understood as the spontaneous breaking of the right-left symmetry of the quarks (chiral symmetry). The problem is to show that in QCD chiral symmetry is actually spontaneously broken.

A new class of gauge field configurations, instantons<sup>28</sup>, have recently been discovered in the classical non-abelian gauge theories and they may provide a better understanding of the quantum theory (for example, QCD). In the quantum theory, instantons imply that tunnelling takes place between topologically distinct vacuum states. There are already suggestions that instantons may be responsible for breaking chiral symmetry in QCD thus elucidating the pion's properties. Instantons may also lead to a two-phase structure in QCD—strong and weak coupling—that could give rise to a bag picture of confinement<sup>29</sup>. Many theorists are trying to solve these problems and elucidate QCD's properties.

## Outlook

QCD and QED (Weinberg-Salam model) are both non-abelian gauge theories. Together they describe strong, weak and electromagnetic interactions. The next natural step is to embed these two distinct theories in a larger compact covering Lie group, for example, SU(5) (ref. 30), and thereby construct a grand unified theory of strong, weak and electromagnetic interactions. Such a theory has only one gauge coupling constant, so all three interaction strengths are equal at very short distances (true unification); however, this unification only becomes apparent at ultra-high energies  $\sim 10^{16}$  GeV. In these types of models leptons and quarks belong to the same group representations. So leptons, such as the electron, are very much like quarks; but they are not strongly interacting and are liberated because they do not carry colour. A novel prediction of many such grand unified models is that baryon number is not absolutely conserved; so the proton may actually decay! (Its half life is  $\tau_p \geq 10^{30}$  yr.) This predicted instability is being looked for experimentally. Ultimately, one wants to bring gravity into this unification scheme; however, in spite of much effort a renormalisable quantum theory of gravity is not known to exist.

For decades high energy theorists have sought a fundamental theory of the strong interaction: many are now convinced that QCD is the answer. It can be explicitly written down as a renormalisable quantum field theory; the problem has become to solve QCD and make further experimental contact. The endurance of the concept of a continuum quantum field theory as a way of understanding physics at  $10^{-13}$  cm and smaller is especially remarkable.

The shift in emphasis from searching for the theory of the strong force to solving it may revitalise the symbiosis of mathematics and physics in the coming decades. Strong interaction physics could become like atomic physics after the Schrödinger equation.

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# articles

## Re-appraisal of lithostratigraphy of Makapansgat Limeworks hominid site

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*A new lithostratigraphic classification is proposed for the Makapansgat Limeworks hominid site. The Makapansgat Formation is defined in terms of constituent members and beds. The new terminology is correlated with that used previously and hominid finds are linked to the newly defined lithostratigraphic units.*

THE stratigraphy of the hominid site at Makapansgat Limeworks, where *Australopithecus africanus* was discovered in association with a rich fauna in 1947, has been described in several publications. Between 1947 and 1957 several broad descriptive classifications were proposed<sup>1-5</sup>. The first detailed stratigraphic and sedimentological analysis appeared in 1958 (ref. 6) and has provided the standard lithostratigraphic subdivision of the deposit until recently. The re-appraisal of the hominid deposit reported here was necessary because earlier studies omitted several important subdivisions within major stratigraphic units. In this re-appraisal I call the hominid-bearing deposit the Makapansgat Formation.

### Location and origin

The Makapansgat Formation comprises a large cave filling at the Makapansgat Limeworks (24°08' S, 29°11' E). It is exposed both at surface and abandoned lime quarries at elevations of 1,440–1,475 m. At the surface the maximum dimensions of the deposit are approximately 200 × 115 m, increasing slightly with depth. The outcrop area is associated with a pronounced bench on the steep southeastern slopes of the Dorps river valley.

The deposition and subsequent modifications of the sedimentary sequence were very similar to those at the Sterkfontein hominid site, described previously<sup>7</sup>. A similar origin, conforming to the model of karst development proposed for the Transvaal<sup>8</sup>, is postulated for the original solutional receptacle at Makapansgat. The Malmani dolomite here dips south at a low inclination; together with joints and small faults, this small dip caused preferential solution and influenced the form of the original cavern<sup>8</sup>. Considerable modifications resulted from later roof collapses. The present water table is controlled by the episodic Dorps river, and is usually below the level of the valley floor, ~15 m below the base of the formation.

Small remnants of the original dolomite roof are preserved over the deposit, but much of its surface has been bevelled by erosion of the hillside. Reconstructions based on the positions and inclinations of the roof segments suggest that the maximum elevation of the original roof reached ~20 m above the present surface. Hence the absolute thickness of the deposit may have approached 50 m in places; less than 60% of this is preserved.

As with the Sterkfontein Formation<sup>7</sup>, the stratigraphic relationships are complex, and the schematic column in Fig. 1 merely reflects the relative average thicknesses of the various units in accessible areas of the deposit.

### Lithostratigraphy

Samples were prepared in the same way as those from Sterkfontein<sup>7</sup>. They were recovered in vertical succession from four areas at an average vertical separation of <2 m; supplementary samples were recovered from other localities to monitor lateral variations in sedimentary facies.

The very coarse (>2 mm) fraction of all units was examined visually. In the lowest members (1–3) it is chiefly bone and broken speleothems, although occasional pebbles and cobbles of dolomite, chert, quartzite and shale occur in Member 3. In Members 2 and 3 much of the bone is stained with pyrolusite, and many of the broken speleothems show post-depositional weathering. In Member 4 the very coarse fraction is predominantly angular cobbles and boulders of chert and dolomite; many individual fragments are fresh on one or two faces with thin weathering skins on others, indicating that they accumulated through intracavernous collapses of the roof and projecting ledges. The finer component includes subrounded chert pebbles and cobbles whose deeper and more uniform weathering is compatible with an extracavernous colluvial source. Occasional weathered quartzite pebbles and cobbles are present. Quartzite and shale horizons are interbedded in the Malmani dolomite on the upper valley slopes to the south-east of the site.

The very coarse fraction of Member 5 consists overwhelmingly of deeply weathered subangular to subrounded dolomite pebbles and cobbles with few chert and quartzite pieces.

**Member 1** is exposed over most of the cave above the irregular dolomite floor, or overlying large collapsed roof-blocks. It consists of 0.5–15.0 m of white banded reddish-brown (5 YR 5/4), recrystallised meso- and macro-crystalline calcite, locally contaminated with silty loam (clayey, sand-silt). It is thickest in two major stalagmitic bosses, one aligned north-south through the Entrance Quarry (see Fig. 2), the other along the south-east wall of the cave. Concentrations of bone, sometimes as articulated partial skeletons, are present in the distal areas; occasional moderately inclined centimetre beds and cross-beds are visible. The evidence suggests that extracavernous components entered through a relatively small opening near the northern end of the cave. CaCO<sub>3</sub> generally exceeds 80% and average  $\phi$  mean is 6.0 ( $\phi$  is the negative logarithm to the base 2 of the particle diameter (mm)). Sediments are poorly sorted<sup>9</sup> and leptokurtic or highly leptokurtic with pronounced positive skewness. Crystalline minerals (in decreasing order of abundance) are quartz, kaolinite and sericite. Typical particulate roundness and sphericity are 0.3 and 0.5 respectively, and conform with those of present external colluvial soils. As in most succeeding units, quartz particles predominate; chert and dolomite grains are infrequent. Pyrolusite and ferric skins are present on a few particles. Contact is abrupt and wavy.

These features suggest episodic subaqueous deposition of the clastic material with illuvial enrichment in fines. Cross-stratification indicates locally channelled flow during episodic



storms, with relatively high water velocities occurring away from high points in the floor near the centre and entrance of the cave. These could have swept carcasses from the entrance into distal recesses.

**Member 2** is exposed to the west and south-west of both the Entrance and the Exit Quarries, where sedimentation occurred penecontemporaneously in two separate depositories separated by a high area in the surface of Member 1. It consists of 2.0–10.0 m of yellowish-red (5 YR 5/8), and, less frequently light to medium red (2.5 YR 6/6 and 4/8) or light reddish-brown (5 YR 6/4), mostly centimetre-bedded and cross-bedded, silty loam (clayey sand-silt), containing frequent broken speleothems, recrystallised calcite lenticles, and with local concentrations of bone, including some articulated partial skeletons in distal areas. Pebble-sized rolled calcite and bone fragments have sometimes been retained in irregularities in the inclined surface of Member 1 near the position of the original cave opening, and marked lateral changes of facies are evident. Occasional lenses of spongy collophanite and oolitic concretions occur near the top of the member, which has a very uniform upper surface at approximately 1,458 m.  $\text{CaCO}_3$  averages 56% and average  $\phi$  mean is 5.3. Sediments are poorly to very poorly sorted and mesokurtic to leptokurtic and samples have a wide range of skewness from negative to positive. Crystalline minerals are quartz, sericite and haematite. Typical roundness and sphericity are 0.1 and 0.5, indicating higher angularity than is characteristic of the present external colluvial soils. A few particles have pyrolusite and ferric skins. Contact is eroded, slightly wavy and abrupt, and truncates the uppermost cross-beds. The sedimentology indicates deposition of this member beneath the fluctuating surface of a water table which reached about 1,458 m, with local flushing of some fines into more distant depositories. Fairly widespread cross-beds with rolled calcite pebbles and bones in the proximal facies indicate channelled flow with relatively high water velocities down the irregular, sloping surface of Member 1. As in Member 1, these flows could have carried carcasses into recesses. The collophanite lenses indicate significant bat populations towards the end of this deposition.

**Member 3** is exposed at the southern end of the cave to the east and west of the Cone area, where it consists of 0.5–2.0 m of abundant bone fragments (some semi-articulated) in a matrix of very pale brown (10 YR 8/3 and 8/4) and brownish-yellow (10 YR 6/6), sometimes blotched reddish-brown (2.5 YR 5/4) and black, sandy to silty loam (slightly clayey silty sand), containing extensive calcite intergrowths, numerous broken speleothems, occasional pebbles of dolomite, chert, quartzite and shale, carrion fly pupae, a few coprolites and frequent pockets of collophanite. Most of the deposition occurred in the stalactite fringe immediately below the dolomite cave roof, and crude stratification is present within the bone fragments between stalactite clusters. Flow diversion around the latter has increased bed-shear and created extensive bone lags showing variable particle orientations.  $\text{CaCO}_3$  averages 67% and average  $\phi$  mean is 4.6. Sediment samples show much variation, but are generally very poorly sorted and platykurtic to leptokurtic with a wide range of skewness from negative to positive. Crystalline minerals are quartz, fluorapatite, sericite and chlorite. Typical roundness and sphericity are 0.3 and 0.5, and a very few particles have pyrolusite skins. Comminuted bone and chert particles are relatively abundant. Where Member 3 is present vertically below Member 4, for example east of the Cone, it is overlain by some 0.5 m of laminated, recrystallised meso- and macro-crystalline calcite. Contact between Member 3 and calcite is slightly wavy and abrupt.

The characteristics of Member 3, which has produced the majority of the fauna and hominid remains, are consistent with rapid alternations in depositional conditions with the occurrence of several short, high-energy cycles, which coincide with large fluctuations in concentration of the isotopes  $^{13}\text{C}$  and  $^{18}\text{O}$  in the calcite fraction of the matrix<sup>10</sup>. Quieter episodes apparently alternated with vigorous flows which carried bone fragments and

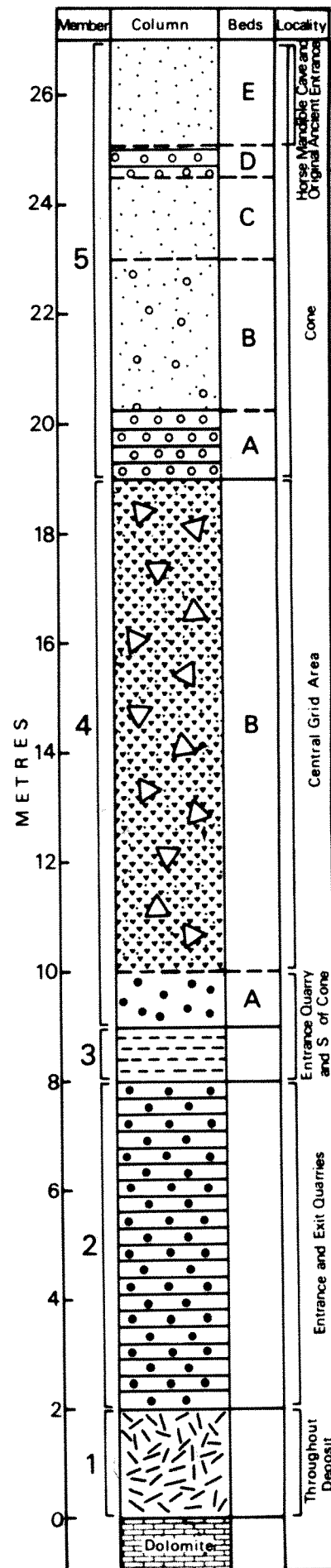


Fig. 1 Stratigraphic column of the Makapansgat Formation.

dismembered carcasses towards the back of the cave and winnowed some finer particles. Between sheetfloods decomposing carcasses attracted carrion flies. The restricted roof height and distance from the cave opening argue against any lengthy occupation of this area by predators or scavengers; such creatures probably concentrated their activities near the cave mouth. Their coprolites could have been incorporated in the deposit during episodes of quieter flow.

**Member 4** consists of beds A and B. Bed A is exposed chiefly in the Entrance Quarry and is composed of 0.5–2.0 m of pink (5 YR 7/4) or light red (2.5 YR 6/6), locally decimetre-bedded, silty loam (clayey silty sand), containing numerous large oolitic concretions, occasional recrystallised calcite lentils and infrequent angular chert and dolomite pebbles and cobbles; some bones and carrion fly pupae are present. Towards the northern end of the Exit Quarry it takes the form of a dense accumulation of rodent bones in a similar fine matrix, which indicates the presence of local owl roost.  $\text{CaCO}_3$  averages 92% and the average  $\phi$  mean is 4.65. Sediments are very poorly sorted and platykurtic to leptokurtic with negative skewness. Crystalline minerals are quartz and sericite. Typical roundness and sphericity are 0.3 and 0.5. Pyrolusite skins are rare. Contact is gradational. These features argue for the deposition and redistribution of sediments in an environment of shallow, localised pools accompanied by winnowing of some fines.

Bed B is present throughout the central part of the cave; it is the most extensive stratigraphic unit, and lime-workings have almost everywhere isolated it from the cave walls. Over substantial areas where the cave floor is high, it overlies either Member 1 or Member 2. It is composed of 2.0–15.0 m of light reddish-brown (5 YR 6/4), less frequently pink (5 YR 7/3), reddish-yellow (5 YR 6/6) or red (2.5 YR 5/8), loam (clayey silty sand), containing abundant broken speleothems, chert and dolomite pebbles, cobbles and boulders, a few quartzite pebbles, occasional bone fragments and recrystallised calcite lentils and void linings. The frequency of chert and dolomite fragments increases upwards, and these display crude stratification parallel to the flanks of a debris cone with its apex near the northern end of the deposit. Highest bone concentrations occur along the cone periphery near the cave walls, suggesting that bones were redistributed toward its margins.  $\text{CaCO}_3$  averages 86% and average  $\phi$  mean is 4.95. Sediments are extremely poorly sorted and samples are mostly mesokurtic, with a wide range from platykurtic to leptokurtic. There is a similar range in skewness from negative to positive, with the latter predominating. Crystalline minerals are quartz, sericite and weakly crystallised montmorillonite. Typical roundness and sphericity are 0.3 and 0.7, indicating greater rounding than is characteristic of present external colluvial soils. Pyrolusite and ferric skins are rare. Contact is eroded and disconformable and is locally complicated by subsidence movements.

The characteristics of this bed are consistent with redistribution of gravelly colluvium and some aeolian material, in admixture with frequent roof collapse debris, down the flanks of a cone of gravitative accretion under the influence of episodic sheetfloods entering through an enlarged cave opening. Higher energy conditions are evident towards the middle of this unit.

**Member 5** consists of beds A–E. All are exposed in conformable sequence around the margins of the Cone. The uppermost unit (bed E) also occurs in isolated pockets to the west of the Entrance Quarry, in the eroded surface of Member 4, and in Horse Mandible Cave. In the Cone area deformation and displacement of an underlying calcite horizon indicates that Member 5 accumulated in a large depression formed by subsidence of underlying deposits into lower cavities; it is likely that similar movements preceded sedimentation in Horse Mandible Cave. Beds A, B and D are gravelly and are often lenticular in form, grading laterally into sandy facies. The latter often preserve carrion fly pupae.

Bed A consists of 1.0–2.0 m of yellowish-red (5 YR 5/6), silty loam or loam (clayey silty sand), containing abundant subangular or subrounded pebbles and cobbles of partially weathered

dolomite, occasional chert and quartzite pebbles, and a few badly damaged bone fragments. Good sub-horizontal stratification is present in the very coarse fraction. A single sample had a  $\text{CaCO}_3$  content of 69% and a  $\phi$  mean of 5.1. The sediments are extremely poorly sorted and mesokurtic with small positive skewness. Crystalline minerals are quartz, sericite, haematite and kaolinite. Typical roundness and sphericity are 0.3 and 0.7, and pyrolusite skins are uncommon. Contact is sloping and fairly abrupt.

Bed B includes 3.0–6.0 m of pink (5 YR 7/4) becoming red (2.5 YR 5/6) where decalcified, unbedded loamy sand (slightly silty sand), containing abundant subangular to subrounded pebbles, cobbles and boulders of partially weathered dolomite, a few chert and quartzite pebbles, and occasional badly damaged bone fragments.  $\text{CaCO}_3$  is about 73% where calcified, but falls to about 50% where calcite cement has been removed by solution; average  $\phi$  mean is 2.1. Sediments are very poorly sorted and mesokurtic with pronounced positive skewness. Crystalline minerals are quartz, feldspar, sericite and kaolinite. Typical roundness and sphericity are 0.3 and 0.5 and pyrolusite and ferric skins are rare. Contact is even and abrupt.

Bed C consists of 2.0–10.0 m of red (2.5 YR 5/8), decimetre-bedded loamy sand (slightly silty sand), with a negligible very coarse fraction. Bones are few and badly damaged. A single sample had a  $\text{CaCO}_3$  content of 39% and a  $\phi$  mean of 2.6. Sediments are very poorly sorted and mesokurtic with pronounced positive skewness. Crystalline minerals are quartz, sericite and haematite. Typical roundness and sphericity are 0.3 and 0.5 and pyrolusite skins are rare; ferric skins are slightly more abundant. Contact is even and abrupt.

Bed D comprises a distinct near-horizontal gravel band which lenses out and bifurcates locally. It consists of up to 1.0 m of yellowish-red (5 YR 5/6), silty loam (slightly clayey silty sand), containing abundant subangular to subrounded pebbles and cobbles of partially weathered dolomite, occasional chert and quartzite pebbles, and a few badly damaged bone fragments. The very coarse elements show good sub-horizontal stratification. A single sample had a  $\text{CaCO}_3$  content of 56% and a  $\phi$  mean of 4.05. The sediments are very poorly sorted and platykurtic with negative skewness. Crystalline minerals are quartz, sericite and haematite. Roundness and sphericity and the presence of skins are akin to those in Bed C. Contact is even and abrupt.

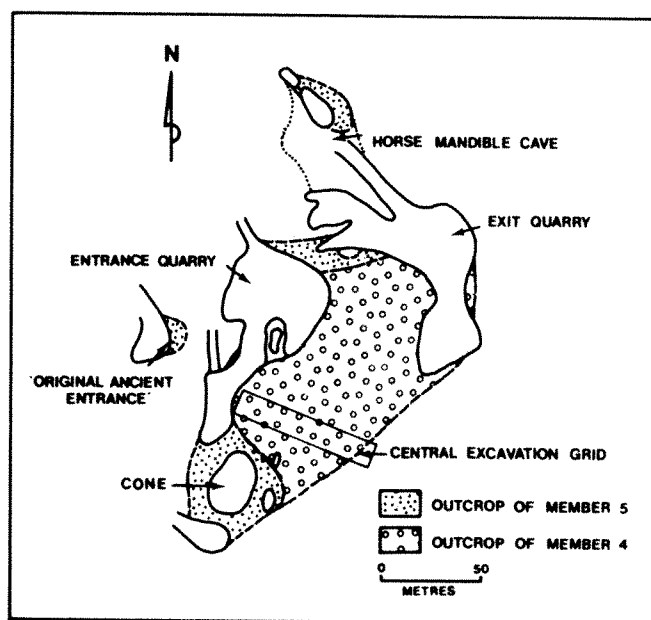


Fig. 2 Locality plan showing the surface extent of the Makapansgat Formation and the positions of localities mentioned in the text.

**Table 1** Comparative terminology of Makapansgat Formation

Previous terminology	Present terminology	Hominid remains
(Brain <sup>6</sup> ) Phase II (red sandy and gravelly) breccia	Member 5	—
Upper Phase I (pink stoney) breccia	Bed A and lower bed B of Member 5 and Member 4	MLD 37/38 from bed B of Member 4
Lower Phase I Grey bone-rich breccia	Member 3	Remainder of specimens of <i>A. africanus</i>
Red calcified muds	Member 2	—
Contaminated travertine	Member 1	—

Bed E consists of 1.0–5.0 m of red (2.5 YR 4/6 and 5/6), and less frequently yellowish-red (5 YR 5/8) and reddish-brown (5 YR 5/4), decimetre-bedded sandy loam (slightly clayey silty sand), with a small very coarse fraction of the type present in the remaining beds, and occasional recrystallised calcite lenticles. CaCO<sub>3</sub> averages 65% and average  $\phi$  mean is 3.8. Sediments are very poorly sorted and samples show a wide range of kurtosis from platykurtic to leptokurtic, but are almost all negatively skewed. Crystalline minerals are quartz, feldspar and sericite. Typical roundness and sphericity are 0.3 and 0.7, indicating greater rounding than is characteristic of the present external colluvium. Pyrolusite and ferric skins are somewhat more numerous than in previous beds.

Viewed as a sequence, the five beds of Member 5 suggest deposition by rapid episodic flushing of unconsolidated gravelly soil from the hillside into depressions containing impermanent pools. The presence of a large proportion of subrounded, weathered dolomite fragments suggests that this gravelly material may have been an alluvial terrace or a mudflow deposit, which had undergone a degree of post-depositional weathering before being washed into the cave. However, the fine matrix certainly includes a large colluvial component. The cave openings seem to have been greatly enlarged after deposition of Bed A. Illuviation of fines from upper to lower beds is evident, suggesting relatively slow and inefficient calcification after cessation of sedimentation. There is distinct evidence for an energy increase in Beds B and C, but damage to bone fragments in conjunction with the presence of stratified gravel lenses suggests the existence of high energy levels during deposition of most of the member. The morphology of Bed B, with its conspicuous lack of stratification indicates a rapid mass movement following renewed subsidence during its accumulation.

### Extracavernous source materials

Comparative analyses of deposits from the vicinity indicate that the –2 mm fraction of the various units was derived mostly from extracavernous colluvial sources except in Member 5, where a significant alluvial (?) component is evident, especially in the very coarse fraction. The contribution of the insoluble residue produced by intracavernous corrosion is chiefly restricted to a few highly angular chert particles.

The present colluvium has relatively uniform properties; it is a pebbly, skeletal soil containing abundant, partially weathered, chert pebbles and cobbles. The characteristics of the Makapansgat Formation can be explained in terms of the reworking of this material. It is a dark reddish-brown (5 YR 3/3 and 3/4) sandy loam (slightly clayey silty sand) containing abundant weathered chert pebbles and cobbles, and seldom exceeding 50 cm thick. The  $\phi$  mean averages 2.8. The material is very poorly sorted and is mesokurtic and samples show a wide range of skewness, both negative and positive. Crystalline minerals are quartz, sericite and kaolinite. Typical roundness

and sphericity are 0.3 and 0.5 and many particles have pyrolusite skins.

In Member 5, while a large colluvial component seems to be present in the fine matrix, the very coarse fraction has many of the characteristics of an alluvial or mudflow deposit.

Sedimentary facies of the various units of the Makapansgat Formation can be explained through the reworking of extracavernous colluvium by a limited suite of transportational and depositional processes. The colluvium contributing to Member 1 has been enriched in fines, while that of Member 2 shows that, although fines have increased overall, as expected with subaqueous deposition at a distance from the cave entrance, some winnowing has taken place, especially in proximal facies produced by rapid channelled flow. Winnowing of fines is even more evident in Member 3, where it seems to have occurred during several cycles of flushing. The source material for Bed A of Member 4 seems to have been subjected to similar, but probably less intense flushing. In Bed B of Member 4 enrichment in fines is widely evident, possibly due to an aeolian component, although local winnowing can be discerned away from the walls of the depository. Some enrichment in fines, evidenced by pronounced positive skewness, has occurred in beds A–C of Member 5, probably due to post-depositional illuviation from the upper beds before calcification of the deposit.

In general, fine sedimentary fractions are more abundant in lower members, due to a greater degree of illuviation with increasing distance from the cave opening. Removal of fines can be attributed to flushing and eluvial effects as sheetfloods spilled into the cave opening(s) and were channelled downwards into more distant caverns.

Extensive hiatuses in clastic deposition within the cave, sometimes associated with calcite accretion, indicate cycles with reduced influx of extra-cavernous sediments. In some instances such episodes were marked by intra-cavernous scouring and the production of erosional unconformities.

### Solution cavities and discrimination between members

Solution cavities, often exceeding 5 m in depth, were formed in the eroded surface of the Makapansgat Formation chiefly in Bed B of Member 4. These contain residual material, produced by decalcification and *in situ* weathering of the surrounding sediments; this is overlain by recent colluvium with a clear transition. The residual material is occasionally bone-bearing, and is similar in its properties to that occurring in solution cavities in the Sterkfontein Formation<sup>7</sup>.

Sedimentological analysis of samples from the various lithostratigraphic units of the Makapansgat Formation reveals that successive members are distinctive in terms of the standard deviation of at least one of the associated quartile and moment measures or of the sedimentological characteristics of their very coarse fractions.

### Comparison with Sterkfontein and Swartkrans Formations

The stratigraphies of the Sterkfontein and Swartkrans hominid sites, ~250 km to the south-west, have been re-appraised recently<sup>7,11,12</sup> and the Sterkfontein and Swartkrans Formations have been defined. Comparison of these formations with the Makapansgat Formation shows that, although similarities exist, each is distinctive in terms of the sedimentology of specific constituent members and its particular sequence of erosional and depositional events. The fauna thus far recovered from Members 1–4 of the Makapansgat Formation has no equivalent at Swartkrans,<sup>13</sup> but is considered broadly similar to that from the Sterkfontein Type Site (Member 4)<sup>13–14</sup>, although no results have as yet been published which distinguish comprehensively between the faunas present in the various subdivisions of the Makapansgat Formation; these clearly span a substantial period. However, Wells<sup>16</sup> believes that important differences

between the Sterkfontein and Makapansgat faunas suggest that that from Makapansgat (mostly the lower members) is the older. Palaeomagnetic evidence from the Makapansgat Formation<sup>17,18</sup> permits the fairly definite conclusion that Member 1 predates 3.32 Myr, while Member 3 predates 2.90 Myr. Faunal age estimates for the Sterkfontein Type Site fall in the range 2 to 3 Myr. More precise correlations between the faunas of the two sites must await further studies of the respective assemblages.

### Correlation with previous terminology and hominid finds

In Table 1 the published stratigraphic terminology of previous workers at Makapansgat Limeworks is cross-referenced to the terminology used here. The table also gives an indication of the known or likely provenance of the hominid remains.

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Excavations undertaken so far have sampled very small volumes of the *in situ* deposits, the remaining faunal and hominid material having been extracted from material discarded on dumps during lime quarrying operations. The vast majority of finds can be correlated with the rich bone accumulation of Member 3.

I have not considered biostratigraphic aspects of the Makapansgat formation here; these, together with the environmental implications of the data, will be discussed elsewhere.

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# A late Proterozoic ophiolite complex at Jabal Ess in northern Saudi Arabia

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*A folded and lightly-metamorphosed allochthonous thrust slice, representing a complete ophiolite succession (Penrose definition), has been mapped in the late Proterozoic shield of Saudi Arabia. This occurrence supports the suggestion that plate-tectonic motions operated during the late Proterozoic.*

The late Proterozoic stratigraphy of the Arabian Shield is fundamental to the question of whether plate-tectonic motions operated in the Precambrian<sup>1,2</sup>. This vast area of igneous rocks (1,200 km N–S and 600 km E–W) comprises about two-thirds plutonic rocks and one-third lightly-metamorphosed lava flows and volcanoclastics. High-grade metamorphic areas are minor. The shield is characterised by radiometric ages from 1,190 to 530 Myr<sup>3–5</sup>. Within it there are many serpentinised bodies and belts<sup>6</sup>; some extend discontinuously for up to 700 km. The term 'ophiolite' has long been used in the description of these complexes<sup>7,8</sup> and more recently some have been more convincingly identified as incomplete ophiolite complexes<sup>9,10</sup> in the sense of the Penrose field meeting of 1974<sup>11,12</sup>. More recently the serpentine belts have been postulated to be ophiolitic sutures<sup>8,9,13,14</sup>, formed by the closure of oceanic basins. These interpretations, amongst others, have led to a proposed mechanism of evolution of the Arabian Shield by the plate-tectonic opening and closing of sialic crust<sup>15</sup> or by the accretion of island arcs<sup>16</sup>. However, a complete ophiolite succession in the sense of the Penrose definition has never been described in the Arabian Shield, although it has been sought for some years. The most detailed study at Jabal al Wask<sup>9</sup> reported serpentinised ultramafic rocks (dunite, harzburgite, wehrlite, pyroxenite and chromitite), cumulate and high level gabbros and a metabasalt-keratophyre volcanic association. Recently an alternative

explanation for some of the serpentine belts both in Saudi Arabia and Egypt has been proposed<sup>17,18</sup>—that they are serpentinised komatiitic sills. We describe here a complete ophiolite succession from a new locality in the northern shield of Saudi Arabia (Fig. 1).

## Stratigraphy of the Jabal Ess area

The three components of the mapped area are (1) a meta-sedimentary sequence; this is concordantly overlain by (2) the Jabal Ess ophiolite complex, which is in turn overlain and fault-separated from (3) an upper meta-volcanic sequence.

The stratigraphic succession of the area from bottom to top is: (1) **The meta-sedimentary sequence:** south of the ophiolite complex is a sequence of lightly-metamorphosed, pale-green sediments. Strata vary from 1 to 30 m thick, with most around 2 m. The common rock type is a green shale, often with well-developed pencil cleavage. Less common are pebbly shales and conglomerates. Pebbles are well rounded and up to 20 cm in diameter. They are usually stretched, but have mainly lost their original identity due to chloritisation, with only chert and gabbro clasts recognisable. Several lenses of *mélange* up to 10 m thick and composed of gabbro and serpentine, are concordantly contained within the meta-sedimentary sequence.

(2) **The Jabal Ess ophiolite complex:** this complex has a minimum exposed thickness of about 3 km, but is very condensed by folding and faulting (Fig. 1b). It has been divided into nine components, which from bottom to top are: (i) *The mélange zone*, which is up to 250 m thick, and discontinuously present along the southern limit of the ophiolite complex. The *mélange* consists of blocks of leucocratic meta-gabbro (containing meta-dolerite dykes), melanocratic meta-gabbro, pillow lava and dolerite in a sheared serpentine host. Some blocks are up to 30 m in diameter. A lens of sheared serpentine from 2 to 10 m



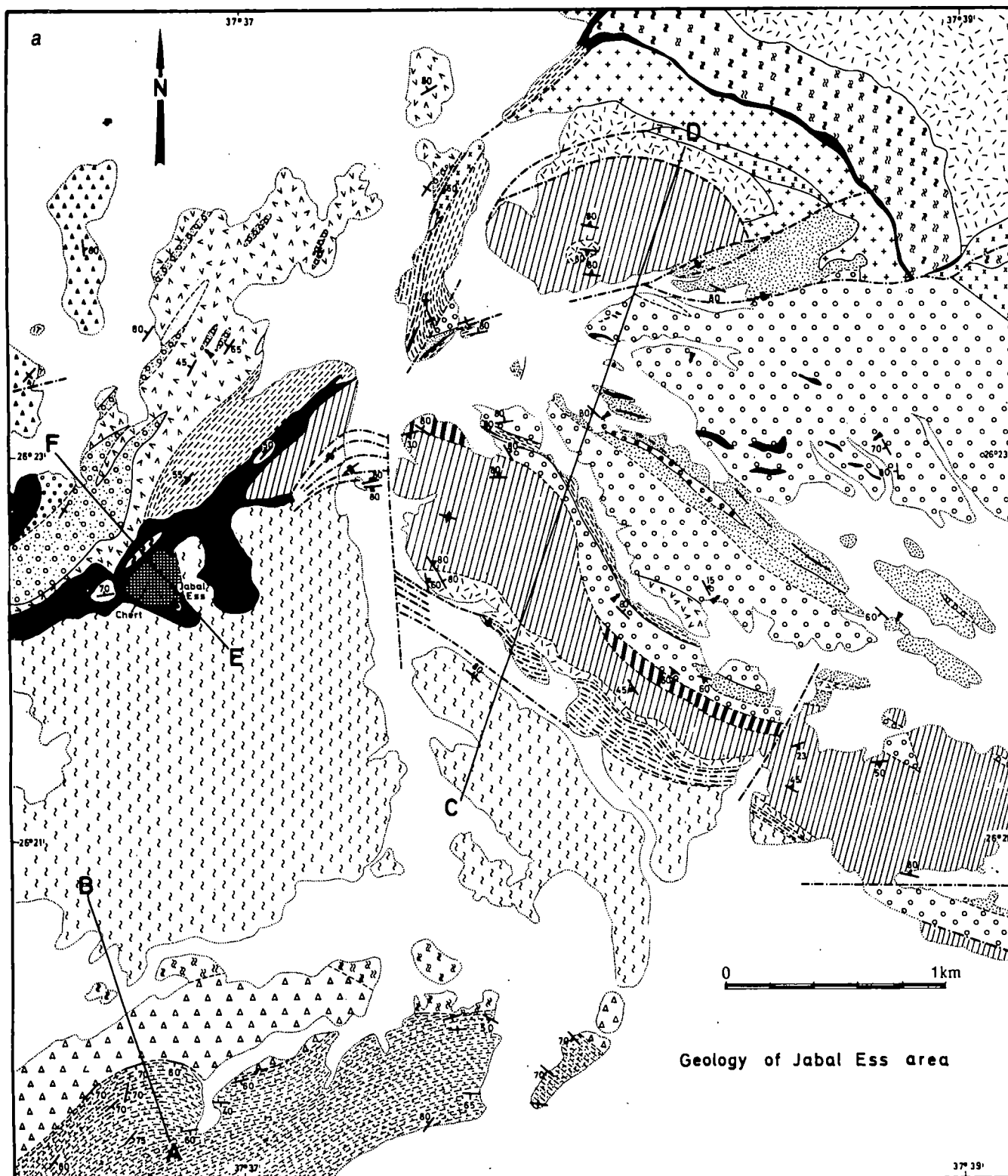
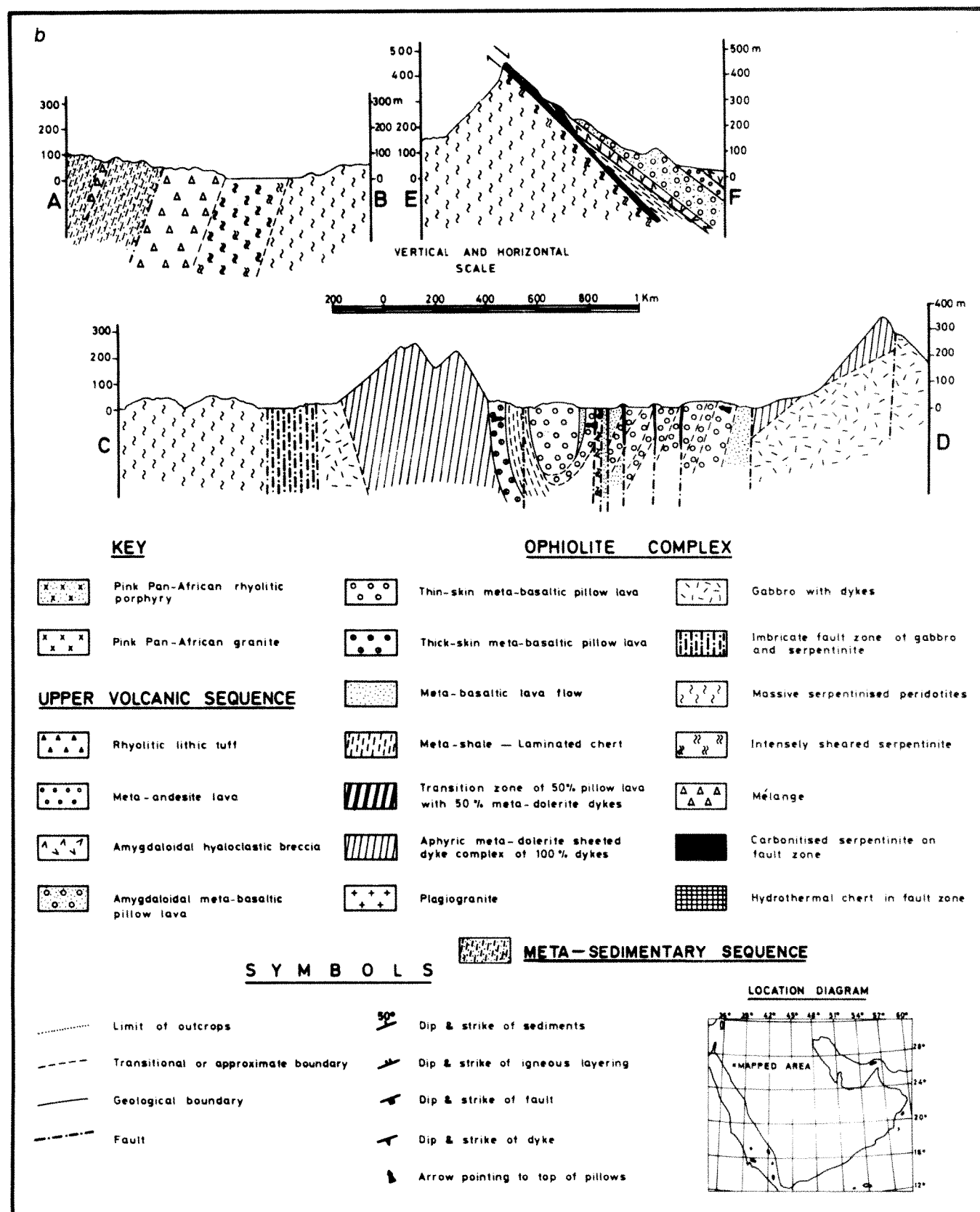


Fig. 1 a, Geological map of the Jabal Ess area, Saudi Arabia; b, (overleaf) cross-sections of the area are shown in a.

wide, which contains blocks of melanocratic meta-gabbro, occurs in direct contact with the meta-sedimentary sequence. Stratification of the meta-sediments and the contact with the mélangé are concordant but folded and overturned in the mapped area. (ii) *Serpentinised metamorphic peridotite*<sup>12</sup>: a black, massive serpentised harzburgite with 5–20% orthopyroxene pseudomorphs shows incomplete serpentisation of olivine, usually with up to 10% relicts. Disseminated chromite is present, as well as podiform chromite lenses up to 20 cm thick. Near the contact with the mélangé, the peridotite is intensely sheared, partly carbonitised and veined by magnesite. (iii) *Serpentinised cumulate peridotite*<sup>12</sup>: the boundary between these

and the serpentised harzburgite has not been mapped in the field. The cumulate peridotites are recognised in thin sections which show serpentised wehrlite with 50% clinopyroxene. Near the imbricate fault zone, thin (up to 30 cm) yellow-weathering layers were seen in the field which proved in thin section to be serpentised dunites. The cumulate peridotite is about 400 m thick adjacent to and south of the imbricate fault zone. The combined thicknesses of the two peridotite zones (ii and iii) is about 1.2 km. (iv) *The imbricate fault zone* is a striking and mappable unit in the field. It is up to 200 m wide and consists of a series of near-vertical fault slices from 1 to 15 m thick of light-coloured meta-gabbro and brown sheared serpentinite.



The meta-gabbro slices rarely show igneous layering and this is truncated by the faults. Usually the meta-gabbro is partly mylonitised so that blocks retaining gabbroic texture are found set in a pale, streaky, mylonitised host. Some of the thinnest fault slices (about 1 m thick) are made up entirely of mylonitised meta-gabbro and lack relict textures. This zone may have faulted out melanocratic layered gabbro as blocks of this rock are

present in the mélange. (v) *Layered gabbro*: a leucocratic gabbro with varying amounts of alteration shows a poorly-developed, rhythmic layering, but clear igneous lamination. Typically areas of pegmatitic-textured gabbro and aphyric meta-dolerite dykes are present. The layered gabbro is poorly represented in the mapped area but occurs within the imbricate fault zone and the sheeted dyke complex. Fault-bounded blocks of gabbro occur in

the north of the mapped area. Many outcrops show all stages of deformation of the gabbro from flaser gabbro to brecciated meta-gabbro set in a mylonitised host. (vi) *The sheeted dyke complex*: most of the complex consists entirely of meta-dolerite dykes. These have a distinctive, aphyric, doleritic texture and a green colour. The distinctive texture allows chilled margins to be recognised. Individual dykes range from 30 cm to 2 m in width. The thickness of this complex varies from 200 to 600 m. The field separation of meta-lavas from meta-dykes is easy as the lavas are much finer grained, contain sparse amygdaloids and weather brown. In the centre of the mapped area, the upper and lower boundaries of the sheeted-dyke complex are transitional over distances of up to 50 m. Along the upper contact some zones of 50% each of dykes and lavas have been mapped (Fig. 1a). Where there is <20% dykes the rocks were mapped as lavas. A striking feature of the upper half of the sheeted dyke complex is the presence of many brown and white patches and areas up to a few metres in size, where the dykes show evidence of metasomatic leaching and iron staining. The orientation of the pillow lavas adjacent to the sheeted dyke complex is near vertical or steeply inclined towards the north. The sheeted dykes immediately below the lavas are orientated at right angles to the lava stratification. Adjacent to the gabbro some of the dykes are now steeply inclined, indicating they were originally at low angles relative to the stratification of the lavas. (vii) *Meta-basalt lavas*: the sheeted dyke complex is overlain by a succession dominated by meta-basalt lavas, at least 300 m thick, but repeated by folding and faulting. Most of the lavas occur as thin-skinned pillow lavas with pale-brown, fine-grained cores and narrow, black-green, chloritised selvages, although a thick-skinned type is sparsely present (Fig. 1). This latter type is characterised by individual pillows having outer chill zones up to 8 cm thick which contain an abundance of white spherulites up to 4 mm in diameter. The cores of this type of pillow are composed of the normal meta-basalt. Almost all of the meta-lavas are aphyric, although one occurrence of plagioclase-aphyric pillow lava was found. Many of the pillows have a tubular form. Some horizons of brecciated pillow lava occur and probably indicate palaeoslopes. (viii) *Shale and laminated chert*: within the meta-basalt sequence, there is a single meta-sedimentary unit up to 50 m thick. This consists of thin-bedded, fissile, khaki-coloured shale with some colour variations which indicate the stratification. Within this unit some shale horizons are siliceous and pass into thin units (<1 m thick) of laminated chert. (ix) *Plagiogranite*: a single arcuate (folded?) and fault-bounded block of plagiogranite is present in the much-faulted northern part of the mapped area (Fig. 1a). The plagiogranite is crosscut by dykes of pink Pan-African granite. The mineralogy is essentially quartz and sodic plagioclase ( $An_{10}$ ). The plagioclase is turbid and there is a small amount of a chloritised mafic mineral. (3) *The upper meta-volcanic sequence* is lightly metamorphosed and consists of andesite, dacite and rhyolite lavas with sparse occurrences of pillowed meta-basalt lavas. The latter are distinct in being highly amygdaloidal in contrast to the very sparsely amygdaloidal pillow lavas of the ophiolite complex. Clastic rocks are abundant in the upper meta-volcanic sequence. Most prominent are rhyolitic lithic tuffs and multilithologic submarine slump deposits containing conspicuous rhyolite blocks up to 1.5 m in diameter. The environment of deposition evidently varied from shallow-water submarine to subaerial as some horizons have shales with mudcracks and at another welded tuffs are present.

## Structure and tectonism

The Jabal Ess ophiolite complex occurs as a syncline with the gabbro-serpentine components repeated in the north and the south of the mapped area (Fig. 1a). The abundance of pillow lava structure has been used to indicate the way up. The study (Fig. 1a and b) showed that the meta-basalt lavas are repeated by folding about the synclinal axis. The ophiolite complex is discordantly fault-separated from the overlying upper meta-

volcanic sequence. The faults are distinct because on Jabal Ess the upper meta-volcanic sequence is juxtaposed onto the serpentinised peridotite and then, towards the east, onto successively higher structural units of the ophiolite complex. The faults are carbonitised and locally silicified. This alteration along faults is best developed on Jabal Ess where up to 20 m of serpentinite have been carbonitised.

The base of the ophiolite complex is the bottom of the mélange zone which is concordant with the meta-sedimentary sequence. The latter concordantly contains lenses of mélange up to 10 m thick. The mélange zone presumably represents an original low-angle thrust fault zone which must have cut across all units of the ophiolite complex, as indicated by the content of blocks within the mélange. The Jabal Ess ophiolite complex is interpreted as an allochthonous fault slice which was emplaced by thrusting to concordantly overlie the meta-sedimentary sequence. Significantly, there are no clasts from the upper meta-volcanic sequence within the mélange. This absence suggests that the discordant set of faults, which mark the upper boundary of the ophiolite complex, postdate the basal thrust of the mélange zone.

The ophiolite complex, together with its host rocks, has been twice folded. Both folding events seem to postdate the emplacement of the allochthonous slice as well as the juxtapositioning of the ophiolite complex and upper meta-volcanic sequence. The first folding formed a tight syncline with an east-west fold axis. A later folding event resulted in more open folds about NNW axes, so that the ophiolite complex has a sigmoidal form. The combination of the two folding events has resulted in near-vertical dips for most of the mapped area and the basal mélange zone of the ophiolite complex is now overturned. Only the main faults are shown on the map (Fig. 1a).

## Conclusions

We have confirmed that Proterozoic ophiolite complexes in the sense of the Penrose meeting do exist in the Saudi Arabian Shield. Metamorphism has not obliterated the original rock features in this occurrence, which can be used as a type locality for Arabian Proterozoic ophiolites. The area is readily accessible by road or light aircraft. The recognition of such units as the aphyric sheeted dyke complex will identify similar rocks in other more tectonised areas of the shield.

The presence of an allochthonous thrust slice of Proterozoic ophiolite within the shield is a strong indication that plate-tectonic motions operated during the late Proterozoic. This conclusion strongly supports an accretionary type of model for the crustal evolution of the large igneous terrain of the Arabian Shield.

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# Modulation of the two promoters of the galactose operon of *Escherichia coli*

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*The gal operon of Escherichia coli is controlled by two independent promoters—one is activated and the other inhibited by cyclic AMP and cyclic AMP receptor protein. The two promoters are modulated, however, by the same operator locus and repressor protein.*

THE galactose (*gal*) operon of *Escherichia coli* consists of three structural genes, *K*, *T* and *E*, with the promoter-operator (*P*-*O*) located at the *E* end of the transcriptional unit<sup>1</sup> (Fig. 1). In wild-type *E. coli* cells, expression of the *gal* genes is inducible by D-galactose or D-fucose<sup>2</sup>. The inducer prevents a specific repressor protein, the product of the unlinked *galR* gene, from binding to the *gal* operator<sup>3</sup>. The operator has been defined genetically by the isolation of *cis* dominant mutations, which map to the right of the *E* gene and make *gal* expression constitutive.

It has been shown both *in vivo* and *in vitro* that the *gal* operon is controlled by two promoters: *P*<sub>1</sub> and *P*<sub>2</sub><sup>4</sup>. The *P*<sub>1</sub> promoter requires cyclic AMP and cyclic AMP receptor protein (CRP) as positive control elements for its activity; *P*<sub>1</sub> is normally responsible for *gal* expression in wild-type cells. The *P*<sub>2</sub> promoter does not need the cyclic nucleotide and the receptor protein and is in fact inhibited by the presence of the two regulatory elements. The *in vitro* startpoints of transcription from the two *gal* promoters, *S*<sub>1</sub> and *S*<sub>2</sub>, are also different and separated by five nucleotides (Fig. 1)<sup>4</sup>. In normal conditions of *in vitro* transcription, that is, in the presence of excess nucleoside triphosphate precursors, transcription from *P*<sub>2</sub>, starting at *S*<sub>2</sub>, largely terminates after completing a trinucleotide leader RNA. The reason for this premature termination is not clear and will be touched upon later.

We report in this article that the synthesis of *gal* enzymes under *P*<sub>2</sub> control, like that under *P*<sub>1</sub>, is also inducible and is under the negative control of the *galR* gene product. The same DNA segment in *gal*, defined by the *O*<sup>c</sup> mutations and by *in vitro* *gal* repressor binding experiments, is required for inhibition of both *P*<sub>1</sub> and *P*<sub>2</sub> activities. The accompanying article<sup>5</sup> shows that the location of the *gal* operator with respect to the *gal* promoters differs markedly from other known promoter-operator structures (Fig. 1).

## Induction of *gal* operon

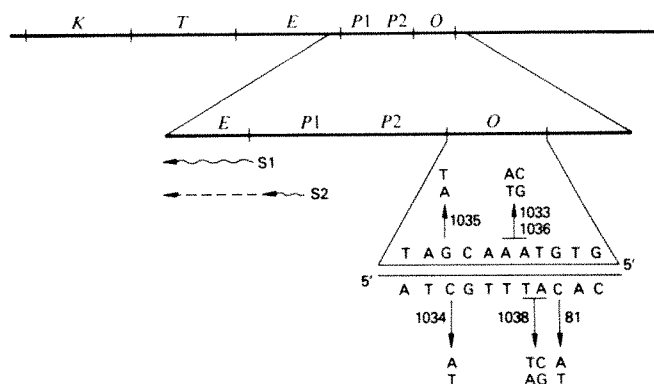
In wild-type *E. coli* cells, under conditions of high cyclic AMP levels, the *gal* enzymes are made almost entirely from the *P*<sub>1</sub> promoter, whereas in cells deficient in cyclic AMP or CRP, the *P*<sub>2</sub> promoter is responsible for the expression of the operon. Thus, the regulatory features of the two promoters can be studied separately simply by measuring the *gal* enzymes made in *cya*<sup>+</sup>*crp*<sup>+</sup> and *cya*<sup>-</sup> (or *crp*<sup>-</sup>) strains, respectively. Evidence that both promoters are not on together *in vivo* in the presence of inducer and cyclic AMP-CRP is provided by the finding that the *P*<sub>1</sub><sup>-</sup> mutation is Gal<sup>-</sup> in *cya*<sup>+</sup>*crp*<sup>+</sup> strains. Table 1 compares the effect of the *gal* inducer D-galactose on the two promoter activities as measured by galactokinase synthesis, the product of the *galK* cistron, in the two strains SA500 (wild type) and SA1039 (*cya*<sup>-</sup>). Clearly, both the promoters are inducible. This is the first experimental evidence that *galP*<sub>2</sub> is inducible; previous measurements of *gal* induction and repression reflected *gal* expression controlled by *P*<sub>1</sub>, the cyclic AMP dependent

promoter. It should be noted that the actual levels of galactokinase, the last gene product, made from a promoter need not reflect the strength of that promoter. It is possible that the two promoters might show different degrees of natural polarity (S. A. and S. Gottesman, in preparation).

However, similar galactokinase induction ratios (about 15-fold) with the two promoters and the fact that both *P*<sub>1</sub> and *P*<sub>2</sub> activities can be induced by the same inducers, D-galactose (Table 1) and D-fucose (data not shown), suggest that *P*<sub>2</sub> is controlled by the same repressor molecule that controls *P*<sub>1</sub>, that is, the *galR* gene product. This was corroborated as follows. The *cya*<sup>-</sup> mutation of SA1039 was introduced into a set of strains, isogenic with wild type strain (SA500), which harbour a series of *galR* mutations. Comparison of galactokinase levels between the corresponding *galR*<sup>-</sup>*cya*<sup>+</sup> and *galR*<sup>-</sup>*cya*<sup>-</sup> strains showed constitutive synthesis in each case, including a *galR* deletion strain (Table 1). The *galR*<sup>-</sup> mutations have previously been shown to be recessive to the *galR*<sup>+</sup> allele<sup>6</sup>.

Identical negative control of both the promoters by the same *gal* repressor was confirmed by studying the effect of a *galR*<sup>S</sup> mutation on *P*<sub>1</sub> and *P*<sub>2</sub>. A *galR*<sup>S</sup> mutation has been shown to produce a non-inducible phenotype in a wild-type background and is dominant over *galR*<sup>+</sup> (refs 1, 7). The phenotype is the result of the altered repressor protein, which fails to interact with the inducer molecule. The *galR*<sup>S</sup><sub>78</sub> mutation retains its phenotype in a *cya*<sup>-</sup> background, that is, galactokinase synthesis remains at the low basal levels both in *cya*<sup>+</sup>*galR*<sup>S</sup> (SA1796) and *cya*<sup>-</sup>*galR*<sup>S</sup> (SA1800) strains in the presence or absence of D-galactose (Table 1).

However, it is conceivable that the non-inducible Gal<sup>-</sup> phenotype of the *cya*<sup>-</sup>*galR*<sup>S</sup> strain is the result of the lack of an effective permease for D-galactose transport into the cell. This



**Fig. 1** Map of the *gal* operon, showing the operator mutations. *K*, *T* and *E* are the structural genes<sup>1</sup>. *P*<sub>1</sub> and *P*<sub>2</sub> are the two promoters, whose positions relative to each other are not known. There may be considerable overlap between the two loci, except that *P*<sub>1</sub> mutations do not affect the *P*<sub>2</sub> activity<sup>4</sup>. *S*<sub>1</sub> and *S*<sub>2</sub> represent startpoints of transcription for their corresponding promoters *P*<sub>1</sub> and *P*<sub>2</sub>. As shown, the *S*<sub>2</sub> transcript frequently terminates after synthesising a trinucleotide. The operator segment shows the nucleotide sequence together with the base pair changes of the six *O*<sup>c</sup> mutations<sup>5</sup>. The map is not drawn to scale.



**Table 1** Effect of repressor on *gal* promoters

Strains		Active promoter	Levels of galactokinase*	
No.	Genotype		-Galactose	+Galactose
SA500	Wild type	<i>P1</i>	0.9	15.7
SA1039	<i>cya</i> <sup>-</sup>	<i>P2</i>	0.6	9.0
SA1857	<i>galR</i> <sub>3</sub> <sup>-</sup>	<i>P1</i>	8.5	7.8
SA1858	<i>galR</i> <sub>3</sub> <sup>-</sup> <i>cya</i> <sup>-</sup>	<i>P2</i>	14.4	11.0
SA1859	<i>galR</i> <sub>B78</sub> <sup>-</sup>	<i>P1</i>	8.3	7.3
SA1860	<i>galR</i> <sub>B78</sub> <i>cya</i> <sup>-</sup>	<i>P2</i>	10.8	7.4
SA1260	<i>galR</i> <sub>Δ</sub>	<i>P1</i>	11.8	12.5
SA1775	<i>galR</i> <sub>Δ</sub> <i>cya</i> <sup>-</sup>	<i>P2</i>	20.6	12.0
SA1796	<i>galR</i> <sub>78</sub> <sup>S</sup>	<i>P1</i>	0.3	0.4
SA1800	<i>galR</i> <sub>78</sub> <sup>S</sup> <i>cya</i> <sup>-</sup>	<i>P2</i>	1.4	0.9
SA1953	<i>galR</i> <sub>78</sub> <sup>S</sup> <i>cya</i> <sup>-</sup> <i>lacP</i> <sub>uv5</sub>	<i>P2</i>	0.7	0.7

Galactokinase was assayed in lysed cells after 2 h of log phase growth in minimal M56 media containing 0.3% D-fructose as carbon source, 0.1% casaminoacids and 0.3% D-galactose as inducer when indicated. Cells were lysed by 0.1 M Tris-HCl, pH 7.9, 0.1 M EDTA and 0.03 M dithiothreitol plus a drop of toluene at 37 °C for 15 min. The wild-type strain, SA500, is F<sup>-</sup> *str*<sup>R</sup> *his*<sup>-</sup> *rel*<sup>-</sup>. All others were derived from SA500. The *galR*<sub>Δ</sub> also deletes the adjacent *lysA* region<sup>14</sup>. The *galR*<sub>78</sub><sup>S</sup> (ref. 1) mutation was introduced into the *galR*<sub>Δ</sub> strain by phage P1 selecting for *lys*<sup>+</sup> and screening for *galR*<sub>78</sub><sup>S</sup>. The galactokinase assay procedure is from Wilson and Hogness<sup>15</sup>.

\*Units: nmol galactose-1-phosphate generated per min by the number of cells equivalent to A<sub>540</sub> of 1.0.

objection was ruled out as follows. Lactose permease, the product of the *lacY* gene, efficiently transports D-galactose<sup>6</sup>. Active *lac* enzymes can be synthesised in a *cya*<sup>-</sup> strain when the *lac* operon carries the *uv5* promoter mutation<sup>8</sup>. Accordingly, the strain *galR*<sub>78</sub><sup>S</sup> *cya*<sup>-</sup> was made into *galR*<sub>78</sub><sup>S</sup> *cya*<sup>-</sup> *lacP*<sub>uv5</sub> to ensure an effective D-galactose transport by selecting for Lac<sup>+</sup> transductant after infection of a bacteriophage P1 lysate made on a *cya*<sup>-</sup> *lacP*<sub>uv5</sub> host. Table 1 shows that similar to *galR*<sub>78</sub><sup>S</sup> *cya*<sup>-</sup> (SA1800) parent, the triple mutant *galR*<sub>78</sub><sup>S</sup> *cya*<sup>-</sup> *lacP*<sub>uv5</sub> (SA1953) is still non-inducible for galactokinase. Taken together, the results with the *galR* mutants convincingly show that the same repressor molecule controls *gal* expression negatively both in the absence and presence of cyclic AMP, that is, both from the *P1* and *P2* promoters.

## The *gal* operator

Using a strain diploid for the *galR*<sup>+</sup> gene, that is, *galR*<sup>+</sup>/*galR*<sup>+</sup>, several *E. coli* mutants constitutive for the *gal* operon were isolated after UV mutagenesis and then enrichment and selection for growth on galactose minimal media in the presence of an anti-inducer of the operon, methyl-β-D-thiogalactoside as previously described<sup>3,6,10</sup>. The *galR*<sup>+</sup> diploid was made by lysogenising wild type strain SA500 with a *λgalR*<sup>+</sup> transducing phage isolated before<sup>9</sup>. Many such mutants carry *cis*-dominant, *gal*-linked constitutive mutations (S.A., unpublished results). These mutations define the *gal* operator (the site of repressor action). Six such independently isolated operator constitutive mutations (*O*<sup>c</sup>), including one that was isolated previously in a *galR*<sup>+</sup> haploid strain<sup>3</sup>, were further characterised as follows:

(1) All six mutations, listed in Table 2, cause very high levels of galactokinase synthesis in the absence of any inducer. The same high levels of galactokinase were made even in the *cya*<sup>-</sup> derivatives of the *O*<sup>c</sup> mutants. If the *gal* operon in the *O*<sup>c</sup> mutants, as in the *O*<sup>+</sup> parent, is transcribed from the *P2* promoter in the absence of cyclic AMP, these results suggest that the same mutations are responsible for making the *gal* expression constitutive both from *P1* and from *P2*.

(2) The levels of galactokinase in four of the six *O*<sup>c</sup> mutants, *O*<sub>81</sub><sup>c</sup>, *O*<sub>1038</sub><sup>c</sup>, *O*<sub>1033</sub><sup>c</sup> and *O*<sub>1036</sub><sup>c</sup>, cannot be further increased by the addition of D-galactose to the growth media under conditions of both *P1* and *P2* activities. This contrasts the *galO*<sup>c</sup> mutants with

*O*<sup>c</sup> mutants in other operons, which usually can be further induced. The behaviour of the *galO*<sup>c</sup> mutants may be attributed perhaps to the demand of the selection procedure used.

(3) The levels of galactokinase in the same four *O*<sup>c</sup> mutants are significantly higher than that in the fully induced *O*<sup>+</sup> cells. This is true, both in *cya*<sup>+</sup> and *cya*<sup>-</sup> backgrounds. The *galO*<sup>c</sup> mutants are, in these respects as well, unique. We have considered three possibilities to explain the hyperactivity of the *galO*<sup>c</sup> mutants: (i) The *galO*<sup>c</sup> mutations have in fact created new cyclic AMP-dependent promoters, transcription from which is not repressible by the *gal* repressor. This may also explain property (2) described above. (ii) The two *gal* promoters and the *gal* operator sequences overlap such that the *O*<sup>c</sup> mutations increase the efficiency of the promoters. (iii) The natural polarity observed in the *gal* operon, that is, synthesis of reduced amount of product from the promoter-distal *K* gene (S. A. and S. Gottesman, in preparation), is eliminated by the *O*<sup>c</sup> mutations in an unknown way. Elimination of natural polarity would increase the levels of galactokinase. *gal* DNA templates, carrying either *O*<sup>+</sup> or one of the six *O*<sup>c</sup> alleles, behave identically in a purified transcription system, that is, they need cyclic AMP for transcription from the *P1* promoter but not from *P2* (refs 5, 11). The latter was always inhibited by the presence of cyclic AMP and CRP. These results do not rule out, but argue against possibility (i). We are now studying possibilities (ii) and (iii).

(4) The accompanying article<sup>5</sup> shows the base-pair alterations of the six *O*<sup>c</sup> mutations in the *gal* control region (indicated in Fig. 1). All six mutations cluster in a small region (spanning seven bases). This segment is located about 60 nucleotides upstream from the start site of the S1 transcript. The short length of the *galO* segment and the fact that two of the mutations, *O*<sub>1033</sub><sup>c</sup> and *O*<sub>1036</sub><sup>c</sup>, show identical base-pair changes and two others (*O*<sub>1034</sub><sup>c</sup> and *O*<sub>1035</sub><sup>c</sup>) affect the same base pair suggest uniqueness of the operator structure. [Note that half of the mutations show simultaneous change of two adjacent base pairs. This may be attributed to the nature of the mutagen used (UV light) to induce the mutations. UV light has been shown to cause similar tandem base-pair changes in other *E. coli* genes<sup>12</sup>.]

## Discussion

The *gal* operon of *E. coli* is unique in that the same structural genes are controlled by two natural promoters. *P1* and *P2* meet the additional requirements of galactose enzymes for anabolic reactions under extreme physiological conditions—excess or deficiency of cyclic AMP. The results discussed above show that both promoters are subject to repression. The same regulatory elements, *gal* repressor, D-galactose and the *gal* operator, control the *gal* expression from both *P1* and *P2*. A contrasting feature of the two promoters is that one (*P1*) is activated and the other (*P2*) inhibited by cyclic AMP and its receptor protein. For *P2*, cyclic AMP and CRP together are not co-repressors acting

**Table 2** Effect of operator mutations on the *gal* promoters

Operator allele	Levels of galactokinase in			
	<i>cya</i> <sup>+</sup>		<i>cya</i> <sup>-</sup>	
<i>O</i> <sup>+</sup>	-I	+I	-I	+I
<i>O</i> <sub>81</sub> <sup>c</sup>	1.0	18.0	0.7	8.1
<i>O</i> <sub>1038</sub> <sup>c</sup>	31.1	36.1	30.5	29.6
<i>O</i> <sub>1033</sub> <sup>c</sup>	32.0	34.3	34.0	32.0
<i>O</i> <sub>1036</sub> <sup>c</sup>	34.0	33.4	36.0	31.3
<i>O</i> <sub>1034</sub> <sup>c</sup>	33.1	34.2	34.6	32.0
<i>O</i> <sub>1035</sub> <sup>c</sup>	9.2	14.9	26.0	29.2
<i>O</i> <sub>1035</sub> <sup>c</sup>	31.2	31.3	15.0	29.4

The *O*<sup>+</sup> strain is SA500 described in Table 1. The strains are isogenic with SA500. The *cya*<sup>-</sup> mutation was transduced into the *O*<sup>c</sup> mutants with phage P1 by selecting for a *val*<sup>R</sup> marker and screening for Lac<sup>-</sup> phenotype. The *val*<sup>R</sup> marker is 40% co-transducible with the *cya*<sup>-</sup> allele (A. Das, unpublished results). The other details are also described in Table 1. -I and +I indicate the absence and presence of D-galactose (inducer) during cell growth.

in concert with the *gal* repressor, because *crp*<sup>-</sup> mutants do not make the *gal* enzymes constitutively. The molecular mechanisms of action of CRP have been explored and are discussed in the accompanying article<sup>5</sup>.

We have shown above that the *gal* repressor represses *gal* transcription from both *P1* and *P2* by acting at the same operator site, as defined by *O*<sup>c</sup> mutations. *In vitro gal* transcription from the *P1* promoter in a purified system in the presence of cyclic AMP and CRP is inhibited by the addition of the *gal* repressor<sup>11</sup>. As expected, the *gal* repressor failed to show repression when the template DNA carried any one of the six *O*<sup>c</sup> mutations discussed above<sup>5,11</sup>. In repressing the *P1* promoter, *gal* repressor acts in a manner competitive with RNA polymerase plus CRP<sup>13</sup>. However, the mechanism of action of *gal* repressor may be different from simple competition, because the operator is located at an unusual site—well upstream from the RNA polymerase or CRP binding site. The synthesis of the

short S2 transcripts made from the *galOP* restriction fragment in the absence of cyclic AMP and CRP (that is, under the control of the *P2* promoter), however, is not inhibited by the *gal* repressor<sup>5</sup>. The *gal* repressor may fail to repress S2 synthesis in the purified transcription system for at least two possible reasons. (1) Lack of an additional element besides the *gal* repressor is needed for repression of *P2* activity. And (2), *gal* transcription from the *P2* promoter is not controlled by inhibition of initiation, but by elongation of the short S2 transcript. The *gal* repressor might act by inhibiting the elongation of S2 RNA.

We are currently investigating these possibilities in order to explain the above discrepancy between *in vivo* and *in vitro* control of the *gal* operon.

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# Unusual location and function of the operator in the *Escherichia coli* galactose operon

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*The operator of the gal operon is located about 60 base pairs preceding the startpoints of the transcription of the two gal promoters. This location contrasts with the location of the operator in other phage or bacterial operons where the repressor binds more closely to the respective transcription initiation sites. Models explaining how the repressor-operator interactions may control the two gal promoters are presented.*

OUR previous studies have shown that two overlapping promoters control the expression of the galactose operon of *Escherichia coli*. *In vitro* transcription experiments of a DNA fragment containing the *gal* operator–promoter region demonstrated that cyclic AMP and its receptor protein (CRP) were required for the activity of one promoter (*P1*), but inhibited transcription at the other promoter (*P2*)<sup>1</sup>. The transcription initiation site for *P2* precedes the startpoint for *P1* by five base pairs in the DNA sequence. We presented arguments which strongly suggest that these two promoters are active *in vivo* under different physiological conditions<sup>1</sup>.

In addition to this dual cyclic AMP–CRP regulation, the *gal* operon is also controlled by the *gal* repressor, the product of the *galR* gene which is unlinked to the *gal* operon<sup>2,3</sup>. Earlier studies had shown that the *gal* repressor binds specifically and with a

very high affinity to *gal* DNA<sup>4</sup> and blocks *gal* transcription *in vitro* with wild-type *gal* DNA but not with *gal* DNA with operator-constitutive mutations<sup>5</sup>. We report here on the effect of the *gal* repressor at each of the two *gal* promoters, and on the unusual location of the repressor binding site.

By sequencing five *gal* operator mutations we show that the repressor binds about 60–65 base pairs upstream from the startpoint for cyclic AMP–CRP dependent transcription. This contrasts with the location of the operator in the *lac*<sup>6</sup>, *trp*<sup>7</sup> and *lambda* operons. In the latter systems the repressor binds much more closely to the respective initiation sites for transcription and probably directly prevents the stable binding of RNA polymerase to the promoters. The location of the *gal* operator suggests that in *gal* the repressor prevents transcription by a different mechanism.

We also show that in our purified *in vitro* transcription system the *gal* repressor inhibits transcription from *P1*; it does not block initiation of transcription at *P2* in the absence of cyclic AMP–CRP and does not prevent the cyclic AMP–CRP dependent inhibition of *P2*. In contrast, *in vivo* results presented in the accompanying article<sup>9</sup> indicate that both *P1* and *P2* are under repressor control. Possible explanations for this discrepancy as well as models for the mechanism of action of the *gal* repressor will be discussed.

## Sequence changes in *galO*<sup>c</sup> mutants

We have examined five *gal*-linked *cis*-dominant mutants which exhibit a high constitutive rate of synthesis of the *gal* enzymes. Several lines of evidence indicate that these *gal* mutations define a site to which the *gal* repressor binds (see also accompanying article<sup>9</sup>). (1) The mutants were selected as capable of growing in

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**Table 1** The presence of a plasmid harbouring a wild-type or mutant *gal* operator affects expression of the chromosomal *gal* genes

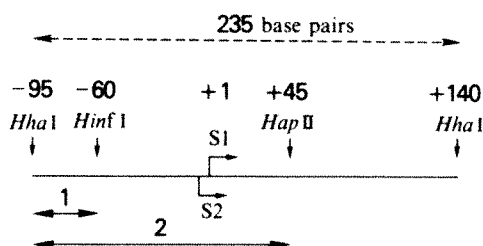
Strain	Genotype of <i>gal</i> operator in plasmid	Galactokinase (units)	
		-D-fucose	+D-fucose
MR94	I	2.8	24.8
MR94/pBdC1	O <sup>+</sup>	18.1	36.9
MR94/pBdC6	O <sup>c</sup>	4.7	20.5

Plasmid pBdC1 is derived from pBR322 in which the 30 base pair fragment between the unique *Eco*RI and *Hind*III sites of pBR322 has been replaced by an *Eco*RI-*Hind*III fragment derived from  $\lambda$ gal8 DNA<sup>21</sup>. The inserted fragment has a size of about 1,000 base pairs and contains the operator-promoter region of the *gal* operon and about 450 base pairs of the first *gal* cistron (B.deC., in preparation). pBdC1 contains a wild-type *gal* operator whereas pBdC6 contains the O<sub>81</sub><sup>c</sup> *gal* mutation (see Fig. 4a). Medium for growth of cells and assay conditions for galactokinase were as described previously<sup>1</sup>. MR94 is a *recA* derivation of MR93 (collection of E. Singer) constructed by C. B. Bruni.

a minimal galactose medium in the presence of the anti-inducer of the *gal* operon, methyl- $\beta$ -D-thiogalactoside (TMG), a property which is also common to *galR* mutants<sup>10</sup>. Presumably, in both types of mutants no stable *gal* repressor-operator complex is formed, thus expression of the operon is constitutive and these cells can grow in a minimal salts medium containing galactose plus TMG. (2) Earlier *in vivo* transcription studies with  $\lambda$  *gal* DNA showed that the *gal* repressor blocks cyclic AMP-CRP dependent *gal* mRNA synthesis (as measured by DNA-RNA hybridisation) with wild-type *gal* DNA but not with the DNA from a *galO*<sup>c</sup> mutant<sup>5</sup>. (3) We have constructed a plasmid (derived from pBR322) which contains the *gal* operator-promoter region and the promoter proximal third of the *galE* cistron (B.deC., manuscript in preparation). Wild-type cells transformed with this plasmid are also capable of growing in minimal galactose plus TMG. The 30 or more copies of *gal* operator titrate the *gal* repressor and cause the constitutive 'escape' expression of the chromosomal *gal* genes. If a *galO*<sup>c</sup> mutation is introduced in the plasmid, the cells are incapable of growing on minimal galactose plus TMG and exhibit a normal induction pattern of *gal* enzyme synthesis (Table 1). We can assume that, unlike the parent plasmid, the plasmid with a *galO*<sup>c</sup> mutation is unable to titrate the cellular *gal* repressor. Hence, the mutation marks the repressor binding site in the *gal* regulatory region.

We have previously established a restriction map for the regulatory DNA segment preceding the first *gal* structural gene<sup>11</sup>. Figure 1 shows the restriction sites of interest. Endonuclease *Hinf* cleaves wild-type *gal* DNA 60/59 base pairs (on the I strand) preceding S1, the startpoint for cyclic AMP-CRP dependent transcription. With two of the mutants (O<sub>1034</sub><sup>c</sup> and O<sub>1035</sub><sup>c</sup>) the DNA is not cleaved by *Hinf* at this site (data not shown), suggesting that these mutations may lie within the recognition site for *Hinf* endonuclease.

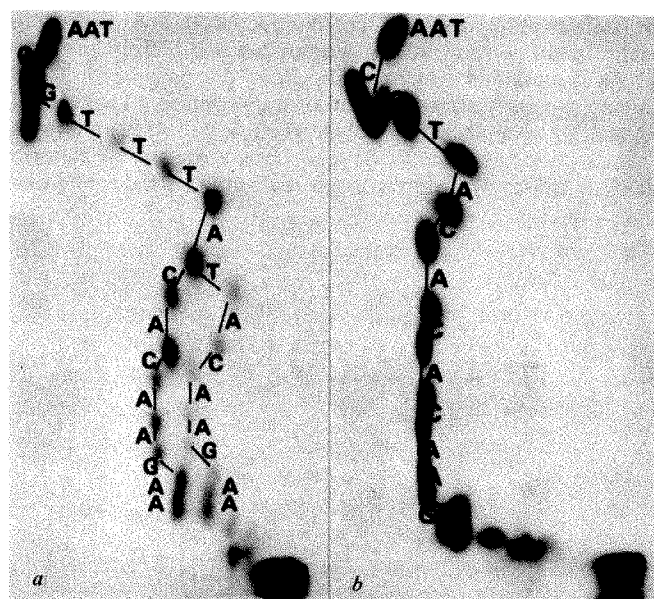
The base changes for each of five mutants were determined. The sequence data are detailed below. The 35 base pair DNA fragment 1 (see Fig. 1) from mutant O<sub>81</sub><sup>c</sup> was labelled selectively at its *Hinf* 5' end and digested by snake venom phosphodiesterase. The partial exonuclease digestion products of the mutant fragment and of a similarly treated wild-type DNA fragment were mixed and fractionated by two-dimensional fingerprint analysis<sup>19</sup>. Figure 2a compares the mobility shifts of the fractionation products of the mutant fragment with those of the wild-type fragment. The patterns deviate at the site of the mutation indicating that the C residue (-66) in the wild-type sequence<sup>19</sup> is replaced by a T residue in the mutant. Immediately following the mutated residue the succession of mobility shifts runs parallel in the mutant and in the wild-type pattern. Hence no other base change occurs adjacent to the first mutation. That the new shift in the mutant is caused by a T residue and not by a G is indicated by analysis of its mobility shift and the mobility



**Fig. 1** Restriction map of the *gal* operator-promoter region. S1 is the startpoint for cyclic AMP-CRP dependent *gal* transcription, S2 is the startsite for cyclic AMP-CRP independent *gal* transcription. 1 and 2 refer to restriction fragments used for sequencing studies. The DNA sequence of the *gal* operator-promoter segment has been determined<sup>19</sup>.

shifts produced by the addition to known T and G residues in the wild-type sequence. Furthermore, when the same fragment I of mutant O<sub>81</sub><sup>c</sup>, also uniquely labelled at its *Hinf* 5' end, was treated with dimethylsulphate (DMS) according to the procedure of Maxam and Gilbert<sup>10</sup> and the fractionation products analysed by gel electrophoresis, it was clear that no new G residue appeared at position -66 (data not shown).

A similar analysis was applied to the DNA of mutant O<sub>1033</sub><sup>c</sup>. The two-dimensional fractionation of the partial nuclease degradation products of fragment I shows that two adjacent T residues in the wild-type sequence (-64 and -63) have been replaced by an A residue (-63) and a C residue (-64) (Fig. 2b). A DMS analysis on the same mutant fragment, labelled at the



**Fig. 2** Partial venom exonuclease digests of fragment 1 (of Fig. 1) terminally labelled at its *Hinf* 5' end from wild type and from the mutants O<sub>126</sub><sup>c</sup> and O<sub>1033</sub><sup>c</sup>. A fragment obtained by *Hinf* cleavage, which contains DNA to the left of the *Hinf* site in Fig. 1, was prepared as in ref. 11. This fragment was purified from  $\lambda$ gal8 DNA<sup>21</sup> (wild type) or its isogenic derivatives containing the O<sub>126</sub><sup>c</sup> or the O<sub>1033</sub><sup>c</sup> mutation. The fragments were dephosphorylated with bacterial alkaline phosphatase, 5'-end labelled with [ $\alpha$ -<sup>32</sup>P]ATP using T4 polynucleotide kinase<sup>19</sup> and digested with endonuclease *Hha*I. The products were resolved on a 10% polyacrylamide gel, and fragment 1 of Fig. 1 was eluted from the gel as described elsewhere<sup>11</sup>. Wild-type or mutant fragment 1 (individually or in combination) was partially digested with venom exonuclease in the presence of trace amounts of DNase I and fractionated by electrophoresis on Cellogel in 8M urea at pH 3.5 followed by homochromatography<sup>19</sup>. a, O<sub>126</sub><sup>c</sup> DNA and wild-type DNA; b, O<sub>1033</sub><sup>c</sup> DNA.



*Hinf* 5' end, confirmed the presence of an A change at -63 (data not shown).

As indicated earlier, the DNAs of mutants  $O_{1034}^c$  and  $O_{1035}^c$  lack the *Hinf* cleavage site at -60/-59 and hence were not amenable to the same partial exonuclease analysis as the preceding mutants. Fragment 2 (*Hha*-1/*Hap*-1) was end-labelled at the 5' *Hha*-end for each of these two mutants and sequenced according to Maxam and Gilbert<sup>12</sup>. Figure 3a compares the fractionation products resulting from the DMS and hydrazine reactions of wild-type DNA and  $O_{1034}^c$  DNA. A single base substitution, A for G, occurs in the mutant DNA at -60. The base pair which is mutated in  $O_{1034}^c$  thus lies within the *Hinf* site.

In mutant  $O_{1035}^c$  the same G/C base pair (-60) is changed to a T/A base pair. Figure 3b shows the products of chemical fractionation of the *Hha*-1/*Hap*-1 fragment (fragment 2 of Fig. 1) for this mutant. Comparison with the wild-type sequence in Fig. 4 indicates the position of the base change.

A similar analysis was performed with the same *Hha*-1/*Hap*-1 DNA fragment of mutant  $O_{1038}^c$  (Fig. 3c). In this mutant two adjacent base pairs G/C and T/A have replaced the two adjacent A/T base pairs at -65 and -64.

Figure 4a summarises the results of the sequence analysis of the five mutants. The mutations which we examined are all clustered in a small segment between -66 and -60.

### Effect of *gal* repressor on *in vitro* transcription

*In vivo* experiments<sup>9</sup> indicate that the interactions between the *gal* repressor and the *gal* operator repress both *P1* and *P2*. We have also studied the effect of *gal* repressor on initiation of transcription *in vitro* at each of the two startpoints. When the 235 base pair *Hha*I fragment (see Fig. 1) is used as template and the transcription reactions are conducted at low nucleoside triphosphate concentrations, a characteristic pattern of small RNAs is obtained which results from the pausing of RNA polymerase early after initiation of transcription<sup>1</sup>. These small RNAs can be analysed by polyacrylamide gel electrophoresis. Transcription initiating at S1 generates a diagnostic pattern, which is strikingly different from the pattern produced when

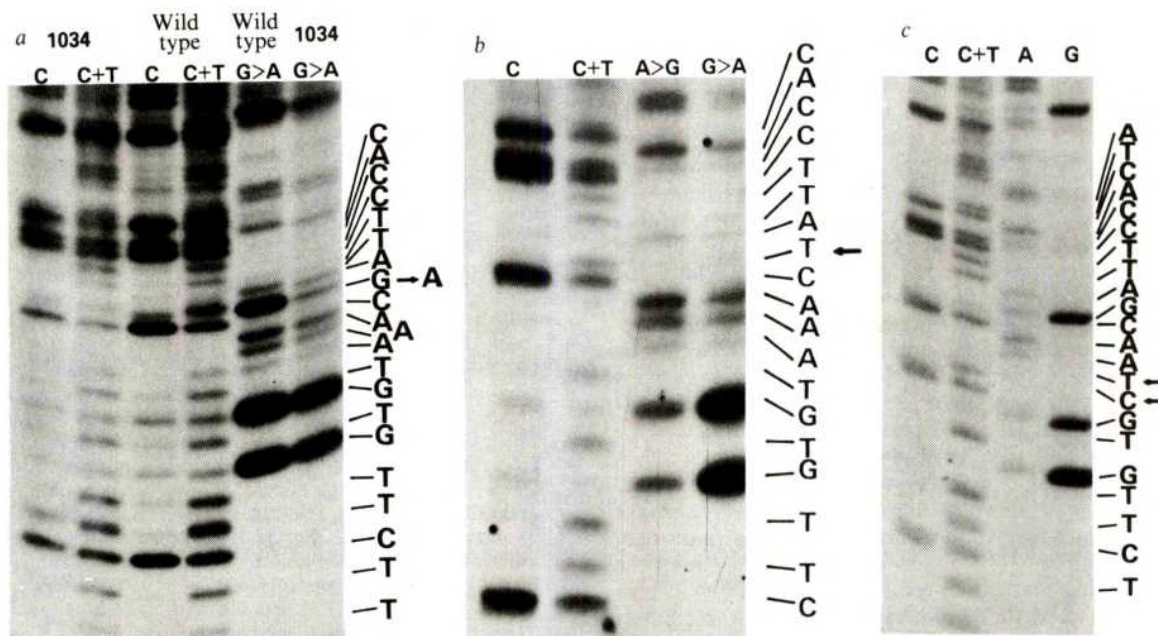
**Table 2** Efficiency of transcription initiation at *P1* and *P2*

	Specific activity per UMP residue	
	in 3-nucleotide-long oligonucleotide	in 9-nucleotide-long oligonucleotide
+cyclic AMP-CRP	—	1,100
-cyclic AMP-CRP	5,700	—

Comparison of the efficiency of initiation at S1 and S2. The 235 base pair *Hha*I fragment of Fig. 1 was transcribed as described in the legend of Fig. 5, both in the absence and the presence of cyclic AMP; the RNA was then fractionated on an 18% polyacrylamide gel. From each lane the major RNA band (corresponding to a trinucleotide in the minus cyclic AMP reaction and to a nonanucleotide in the plus cyclic AMP reaction) was located by autoradiography, excised from the gel and counted. The trinucleotide and the nonanucleotide each contain two UMP residues.

transcription starts from S2. In the presence of cyclic AMP-CRP the majority of the *gal* transcripts pause at several points within the first nine bases of the 5' end of the cyclic AMP-CRP dependent *gal* mRNA<sup>1,22</sup>. In the absence of cyclic AMP-CRP, transcription from S1 is completely inhibited and instead is initiated five bases upstream and pauses within a different six-base sequence. The nucleotide sequence of the various short transcripts was previously established<sup>1</sup>. Figure 5 shows that in the absence of cyclic AMP and CRP the *gal* repressor does not cause inhibition of transcription (lane 2). Hence in the conditions of our *in vitro* reaction, the *gal* repressor is unable to prevent initiation of transcription from S2. This conclusion is also valid when transcription reactions are conducted at high nucleoside triphosphate concentrations (data not shown). We have in fact observed a small but consistent increase in S2 RNA synthesis by *gal* repressor preparations (Fig. 5 lane 2). This increase is reversible by D-fucose.

In the presence of cyclic AMP and CRP, however, the *gal* repressor blocks S1 transcription and this repression is overcome by the *gal* operon inducer D-fucose. Although the *gal* operator is located in a segment which shows striking sequence



**Fig. 3** Autoradiographs of DNA sequencing gels. Fragment 2 of Fig. 1 from either wild-type DNA or from the mutant DNAs  $O_{1034}^c$ ,  $O_{1035}^c$  or  $O_{1038}^c$  was <sup>32</sup>P-labelled at its 5' *Hha* end and was subjected to base-specific chemical degradation according to Maxam and Gilbert<sup>10</sup>. The reaction products were fractionated on 20% polyacrylamide gels in 7M urea. The arrows indicate the site of the mutations. a, Comparison of wild-type DNA and  $O_{1034}^c$  DNA; b,  $O_{1035}^c$  DNA; c,  $O_{1038}^c$  DNA.



similarities and lies at the same distance from the initiation site as the CRP binding site in the *lac* operon, addition of repressor does not prevent the cyclic AMP–CRP dependent inhibition of transcription from S2 (Fig. 5, compare lanes 4 and 5). Our experiments thus clearly show that if both the *gal* repressor and cyclic AMP–CRP are present the two *gal* promoters are strongly inhibited.

### Transcription of *gal* operator constitutive mutants

The isolation of mutants which do not bind cyclic AMP–CRP<sup>13</sup> as well as direct chemical protection experiments<sup>14</sup> have defined the CRP binding site in the *lac* operon ( $\sim -70$  to  $-50$ ). Examination of a similarly located segment in *gal* reveals important similarities both in sequence and in symmetry (see Fig. 4b). Since all *galO<sup>c</sup>* mutations which we examined lie within this region ( $-66$  to  $-60$ ), we determined whether these mutations affected the cyclic AMP–CRP regulation of one or the other *gal* promoter. With every *galO<sup>c</sup>* mutant transcription from S1 is still completely dependent on cyclic AMP–CRP, and transcription at S2 only initiates in the absence of cyclic AMP–CRP (data not shown). One *gal* mutant (*galO<sub>81</sub><sup>c</sup>*) was particularly interesting to study since the same base change G/C to A/T at  $-66$  occurs in a *lac* mutant<sup>13</sup> at exactly the same location with respect to the startpoint of transcription in *lac* and S1 in *gal*. These mutations in *lac* and *gal* also occupy the same location within a similar symmetrical sequence (see Fig. 4) that seems to be the CRP binding site in *lac*. In *lac* the mutation causes severe reduction of promoter activity. In *gal* the same mutation results in the constitutive expression of the *gal* operon *in vivo* both in the presence or absence of CRP or cyclic AMP. *In vitro* the CRP concentration dependence and the cyclic AMP concentration dependence for both *P1* activation and *P2* repression are identical whether *galO<sub>81</sub><sup>c</sup>* DNA or wild-type DNA are used as a template. Figure 6 shows the effect of cyclic AMP concentration of *P1* and *P2* transcription for both types of DNA. Hence the mutation does not affect cyclic AMP–CRP function in *gal* in contrast to its profound effect on CRP action in *lac*.

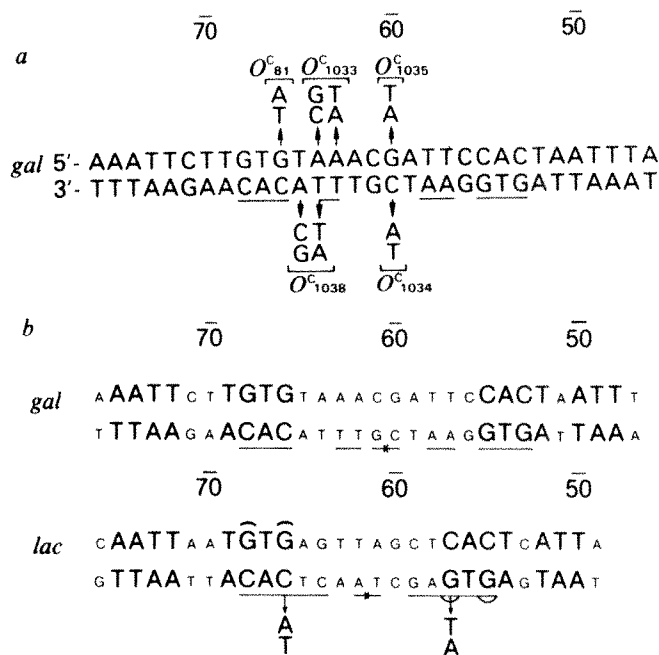


Fig. 4 a, Summary of the base changes caused by 5 different *galO<sup>c</sup>* mutations. b, Comparison of the *lac* CRP site with a similarly located segment in *gal*.

### Initiation at *P2* is more efficient than initiation at *P1*

In an effort to characterise the *P2* promoter further, we compared the efficiency of initiation of transcription at S1 and S2. The characteristic small RNAs corresponding to each promoter as well as the larger RNA, which extends to the end of the fragment, were eluted from the polyacrylamide gel. The RNA sequence of each of the small RNAs is known, and their relative molar yields were determined from the radioactivity of the eluted material ( $[\alpha\text{-}^{32}\text{P}]\text{UTP}$  was used in these experiments). The results shown in Table 2 indicate that in our experimental conditions transcription initiates at S2 about five times more efficiently than at S1.

### Transcription of *P1* and *P2* at high nucleoside triphosphate concentrations

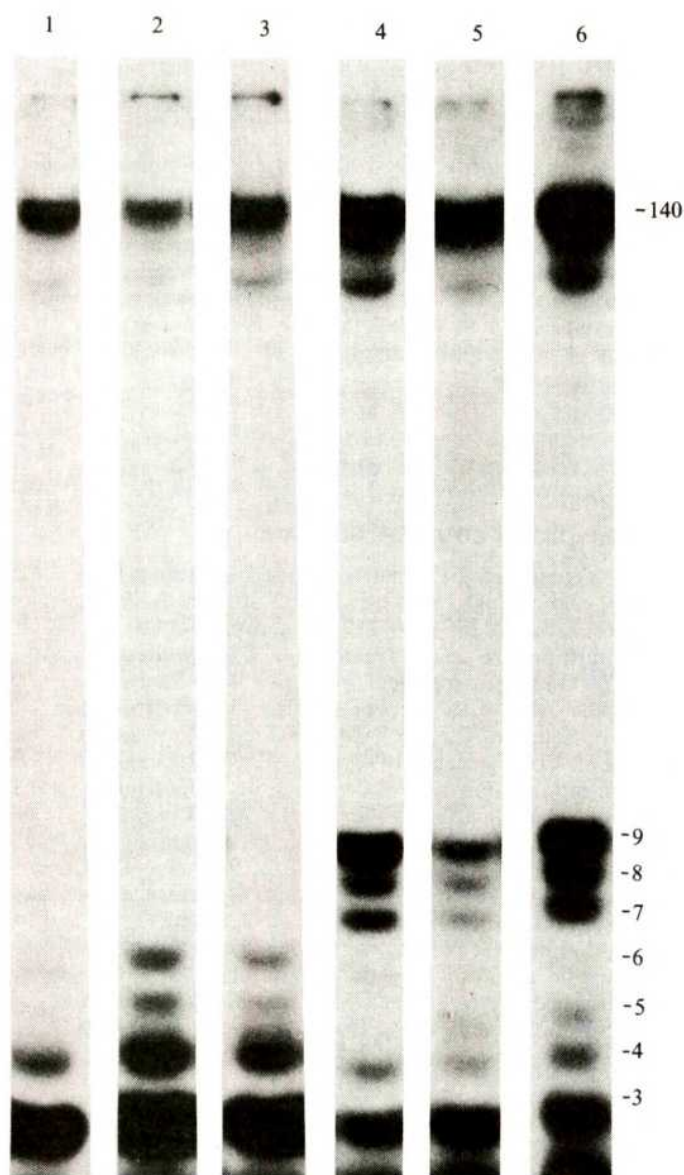
We also compared the pattern of transcription from *P1* and *P2* at low and at high nucleoside triphosphate (NTP) concentrations. If high (150–200  $\mu\text{M}$ ) instead of low (10  $\mu\text{M}$ ) NTP concentrations are used for *in vitro* *gal* RNA synthesis from the *Hha*-230 base pair fragment, stuttering is no longer observed for cyclic AMP–CRP dependent transcription from S1: all transcripts are of large size and extend to the end of the fragment. In the absence of cyclic AMP–CRP using high NTP concentrations, however, a considerable proportion of transcripts originating at S2 still terminates within the sequence of the first 6 or 7 bases (Fig. 7). Hence the termination of transcription from S2 is not just a result of the low NTP concentrations, unlike the pausing observed for S1 transcripts, but may represent an additional regulatory mechanism. Figure 7 also shows that in these conditions, when glycerol was present, less termination and more read-through to the end of the fragment are observed. Glycerol was shown previously to stimulate *gal* transcription from S2 in the absence of cyclic AMP–CRP<sup>1,15</sup>.

### Functional interactions between cyclic AMP–CRP, *gal* repressor and *gal* DNA

The DNA sequencing results presented in this article mark the location of the *gal* operator. The base changes produced by five *O<sup>c</sup>* mutations which we examined all lie between residues  $-60$  and  $-66$ . We have summarised the evidence which indicates that these mutations alter the binding of *gal* repressor to *gal* DNA. Our data do not determine the extent or the size of the operator. A more complete definition of the *gal* operator will be obtained by direct protection experiments against chemical modification of the DNA and by the isolation and sequencing of additional mutants. Such experiments are in progress. Interestingly, in three of the mutants two adjacent base pairs have been changed. The high frequency of this type of mutation is probably due to the method of mutagenesis—UV light.

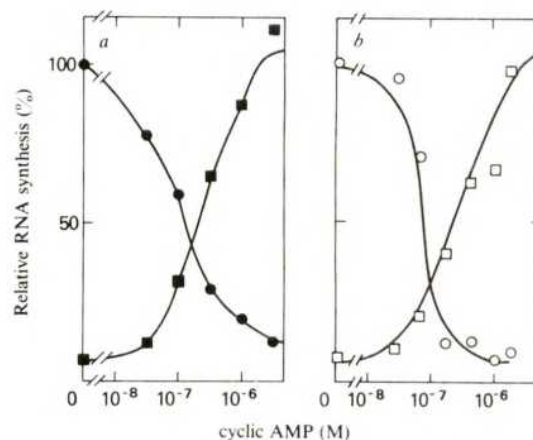
The location of the *gal* operator contrasts with the location of the operators in other operons. The *lac* repressor protects about 20–22 base pairs, between  $-3$  and  $+20$  (ref. 6). The *trp* repressor interacts with a segment which includes at least the portion between  $-5$  and  $-17$  (ref. 16). At each of two  $\lambda$  promoters,  $\lambda P_R$  and  $\lambda P_L$ , the *lambda* repressor binds with decreasing affinity to three adjacent operators. The  $\lambda$  operators which are closest to the respective initiation sites have the highest affinity for the repressor<sup>8</sup>. In each of these systems the repressor thus binds at close proximity to the transcription initiation site and thereby prevents directly RNA polymerase from forming a stable preinitiation complex. The location of the *gal* operator suggests a different mechanism for repression.

Examination of the CRP binding site in *lac* and a segment in *gal* located about the same distance from the cyclic AMP–CRP



**Fig. 5** Effect of the *gal* repressor on initiation of transcription at S1 and S2. The *gal* repressor was purified from an *E. coli* strain containing the plasmid pGR4, a derivative of plasmid pBR313<sup>18</sup> in which the *galR* gene was introduced by *in vitro* recombination (R. di L., unpublished results). The repressor was purified according to a published procedure<sup>5</sup>, except that the affinity chromatography step was substituted with chromatography on single stranded DNA cellulose (R. di L., unpublished). Transcription reactions were carried out in a final volume of 50  $\mu$ l containing 1  $\mu$ mol Tris-HCl (pH 7.9), 5  $\mu$ mol KCl, 0.15  $\mu$ mol MgCl<sub>2</sub>, 5 nmol EDTA, 5 nmol DTT, 2.5  $\mu$ g bovine serum albumin, 0.9  $\mu$ g CRP, 0.5  $\mu$ g RNA polymerase, and 20 ng of the 235 base pair *Hha*I fragment of Fig. 1. After 10 min of incubation at 37 °C, heparin 5  $\mu$ g was added to inactivate unbound RNA polymerase and transcription was started 1 min later by adding 5  $\mu$ l of a solution containing 1 mM ATP and 100  $\mu$ M each of GTP, CTP and [ $\alpha$ -<sup>32</sup>P]UTP (10–20 CI mmol<sup>-1</sup>). Where indicated, cyclic AMP (10 mmol), *gal* repressor (0.9  $\mu$ g) and D-fucose (1  $\mu$ mol) were added before the addition of heparin. After 10 min at 37 °C reactions were terminated by adding 0.25 ml of 0.1 M Tris-HCl pH 7.4, 0.2% SDS, 10 mg ml<sup>-1</sup> tRNA, and 10 mM EDTA. After extraction with phenol, the RNA was precipitated with ethanol, redissolved in 10 M urea, 0.025% bromophenol blue, 0.025% xylene cyanol FF and electrophoresed on an 18% polyacrylamide gel in 7 M urea<sup>11</sup>. Lanes 1–3: no cyclic AMP added; lane 1, no repressor; lane 2, repressor alone; lane 3, repressor and D-fucose. Lanes 4–6: cyclic AMP added; lane 4, no repressor; lane 5, repressor alone; lane 6, repressor and D-fucose. Numbers on the right of the figure indicate the size of the RNAs in nucleotides.

startsite of transcription shows striking similarities both in sequence and symmetry (see Fig. 4b). In *lac*, sequence data of CRP site mutants and chemical protection experiments indicate that the symmetry in Fig. 4b is probably recognised by CRP. In *gal* all *O*<sup>c</sup> mutants which we examined are clustered between –66 and –60, so lie within the same region. Hence one possible mechanism for *gal* repression could imply an interaction of the *gal* repressor with the same site which is recognised by CRP: the *gal* repressor could block *P1* transcription by simply preventing CRP binding to DNA. The following results, however, indicate that despite the similarities in sequence and symmetry CRP does not interact with this segment in *gal*. (1) All *O*<sup>c</sup> mutations which we have examined exhibit the same degree of stimulation of *P1* activity by cyclic AMP–CRP and inhibition of *P2* function by these factors as wild-type DNA. More quantitative data were

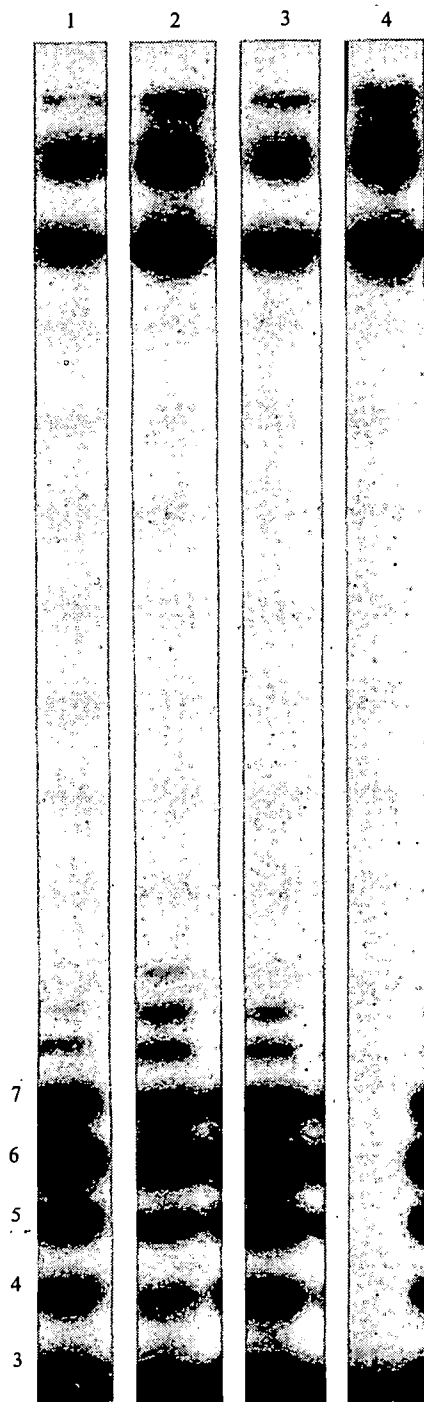


**Fig. 6** Comparison of cyclic AMP effects on *P1* and *P2* transcription for wild-type *gal* DNA and mutant *O*<sub>81</sub>*gal* DNA. Conditions for transcription and analysis of the transcripts on 18% polyacrylamide–7 M urea gels were as described in Fig. 5 legend. Radioactive bands, visualised by autoradiography specific either for *P1* or *P2* transcription, were excised from the gel and counted. A 9-nucleotide-long transcript is specific for *P1*, whereas a 6-nucleotide-long transcript is specific for *P2*. *a*, Wild-type *gal* DNA: ■, *P1* RNA; ●, *P2* RNA. *b*, *O*<sub>81</sub>*gal* DNA: □, *P1* RNA; ○, *P2* RNA.

obtained with one *galO*<sup>c</sup> mutant (*O*<sub>81</sub>). This mutation lies at the same location within the 'common' symmetry and causes the same base change as a *lac* CRP binding site mutation<sup>13</sup> (see Fig. 4). In the mutant *gal*, the cyclic AMP–CRP concentration dependency for *P1* activation (and for *P2* repression) is indistinguishable from that obtained with wild-type DNA. Thus a similar mutation which severely reduces CRP-dependent promoter activity in *lac* has no effect on this function in *gal*, indicating that unlike in *lac* this site is not essential for CRP function in *gal*. (2) Protection experiments indicate that CRP binds between –50 and –25 in *gal* DNA and not to the segment which is analogous to the *lac* CRP binding site (T.T. and B.deC., unpublished results). (3) Replacement of the DNA to the left of –60 with an unrelated DNA sequence does not change the concentration dependency for cyclic AMP–CRP stimulation of *P1* and repression of *P2* (T.T. and B.deC., unpublished results). Thus CRP is capable of activating gene transcription by interacting at a different location in different CRP-dependent promoters.

Our results clearly indicate that binding of the *gal* repressor to *gal* DNA does not prevent the inhibition of *P2* transcription by cyclic AMP–CRP. One possible explanation would postulate the existence of two binding sites for cyclic AMP–CRP, one for

*P1*-stimulation, the other for *P2* repression. *gal* repressor would interfere with the binding of cyclic AMP-CRP at one site, not at the other. An alternative hypothesis to explain both the stimulatory and inhibitory effects of cyclic AMP-CRP is that the



**Fig. 7** Effect of nucleoside triphosphate (NTP) concentrations on transcription from S1 and S2. Transcription reactions and polyacrylamide gel analysis were as in Fig. 5 except for the NTP concentrations as described below. [ $\alpha$ - $^{32}$ P]UTP ( $10$ – $20$  Ci mmol $^{-1}$ ) was used as radioactive precursor. Lanes 1 to 3: no cyclic AMP added. Lane 1: ATP  $100$   $\mu$ M, GTP, CTP and UTP each  $10$   $\mu$ M; lane 2: ATP, GTP, CTP and UTP each  $100$   $\mu$ M and  $20\%$  glycerol (v/v); lane 3: ATP, GTP, CTP and UTP each  $100$   $\mu$ M; lane 4: cyclic AMP added, ATP, GTP, CTP and UTP each  $100$   $\mu$ M.

interaction of cyclic AMP-CRP with a unique DNA binding site could create different protein-protein interactions which would facilitate the formation of a stable transcription initiation complex at *P1* and inhibit this reaction at *P2*. Our finding that the concentrations of both CRP and of cyclic AMP needed for half maximal stimulation of *P1* are identical to those which cause  $50\%$  repression of *P2* is consistent with the latter model. If we postulate such a unique and specific binding site for CRP, it is obvious that the *gal* repressor cannot simply prevent CRP from binding to this site, since CRP still inhibits *P2* transcription in the presence of *gal* repressor. We believe that *gal* repressor could block the productive interaction between CRP and RNA polymerase at *P1* without affecting the interaction between CRP and RNA polymerase at *P2*. We cannot exclude, however, that cyclic AMP-CRP interacts with RNA polymerase first and the cyclic AMP-CRP-RNA polymerase complex binds to *P1* requiring little or no specific interactions between cyclic AMP-CRP and DNA. The same cyclic AMP-CRP-RNA polymerase complex would be unable to bind to *P2*. The high specificity of binding of the cyclic AMP-CRP complex itself to *lac* DNA<sup>12,15</sup> and to *gal* DNA (T.T. and B.deC., unpublished observations) does not favour such an hypothesis.

*In vivo* the *gal* operon can be induced by galactose or fucose even when cyclic AMP or functional CRP are eliminated from the cells by mutation. Both *galR* $^{-}$  mutants and cells harbouring many copies of a plasmid which contains an intact *gal* operator exhibit high levels of galactokinase synthesis whether cyclic AMP or CRP are present or not (see accompanying article<sup>9</sup> and B.deC., unpublished observations). *galO* $^{c}$  mutants also show a high level of constitutive *gal* expression in the presence or absence of cyclic AMP-CRP. These *in vivo* results clearly indicate that the interaction between the *gal* repressor and the *gal* operator causes repression of both *P1* and *P2*. *In vitro*, however, the *gal* repressor blocks only the cyclic AMP-CRP dependent transcription from S1 but does not decrease initiation of transcription from S2. The repressor binds to *gal* DNA in the absence of cyclic AMP-CRP but does not prevent RNA polymerase from initiating transcription at S2. Hence, the *in vitro* results reproduce the *in vivo* regulation by the *gal* repressor when transcription starts from S1. In the absence of cyclic AMP or CRP, however, repression of *gal* transcription occurs *in vivo* but not, in our experimental conditions, *in vitro*. This could suggest that our defined *in vitro* system is lacking one or more elements responsible for the repression at S2 *in vivo*.

In earlier *in vitro* experiments using  $\lambda$  *gal* DNA as template it was found that cyclic AMP and CRP stimulated *gal* mRNA synthesis  $5$ – $10$ -fold<sup>16</sup>. *gal* mRNA was measured by DNA-RNA hybridisation and the majority of the RNA molecules which were analysed were of large size. We have shown here that initiation of *gal* transcription at S2 is in fact more efficient than at S1, but that even with elevated NTP concentrations RNA polymerase terminates transcription prematurely. Thus the low amounts of hybridisable *gal* mRNA found *in vitro* in the absence of cyclic AMP and CRP were probably due to a defect in elongation and not of initiation of transcription. Hence the high levels of *gal* expression which are observed *in vivo* in the absence of cyclic AMP or CRP could be due to a mechanism which would prevent premature termination of transcription. We propose that repression of *gal* transcription from S2, as is observed *in vivo*, could control the rate of premature termination.

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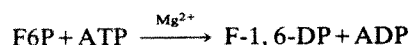
# Structure and control of phosphofructokinase from *Bacillus stearothermophilus*

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*The allosteric enzyme phosphofructokinase binds its substrate fructose-6-phosphate between two subunits of the tetramer, and allosteric effectors between another pair of subunits. The effector binding site accommodates both the activator and the inhibitor. The substrate cooperativity and allosteric control are mediated by these ligand bridges between subunits.*

PHOSPHOFRUCTOKINASE (EC 2.7.1.11) catalyses the phosphorylation of fructose-6-phosphate (F6P) by ATP to form fructose-1,6-diphosphate in a reaction which controls the rate of glycolysis in the cell.



The enzyme has been isolated from a wide variety of sources (for reviews, see refs 1,2), and its activity is regulated by a variety of intracellular metabolites, depending on the source. The enzymes from muscle and from yeast have complex oligomeric structures with subunits of molecular weight 85,000 (refs 1,2) and 100,000 (ref. 3) respectively. In contrast, the enzymes from bacteria are much smaller, and have simpler control properties.

We have worked on the phosphofructokinase (PFK) from the thermophilic bacterium *Bacillus stearothermophilus*, which is a stable tetramer of identical subunits each of molecular weight 33,900. The complete amino acid sequence of 316 residues has been determined<sup>4</sup>. Preliminary kinetic analysis shows a sigmoidal dependence of activity on the concentration of the substrate F6P, allosteric activation by ADP, and inhibition by phosphoenolpyruvate (PEP) (H. Hengartner, unpublished). This allosteric behaviour is similar to that found for the enzyme from *Escherichia coli*, which has been explained with two states of the molecule, an active R-state and a less active T-state<sup>5</sup>. Both enzymes behave as K-systems, that is the two states differ in their affinity for the cooperative substrate F6P, but not in their catalytic rate  $k_{\text{cat}}$ . Unlike the eukaryotic PFKs, these bacterial

enzymes are not inhibited by ATP or citrate, nor activated by AMP. The relationship between the enzymes from prokaryotic and eukaryotic sources remains unclear. We have been looking for a stereochemical explanation of the enzyme activity and investigating the cooperativity of binding of F6P and the influence of the allosteric effectors on the activity. We present here a preliminary report on the structure and substrate binding to the enzyme, which suggests explanations for some of these phenomena.

## X-ray structure determination

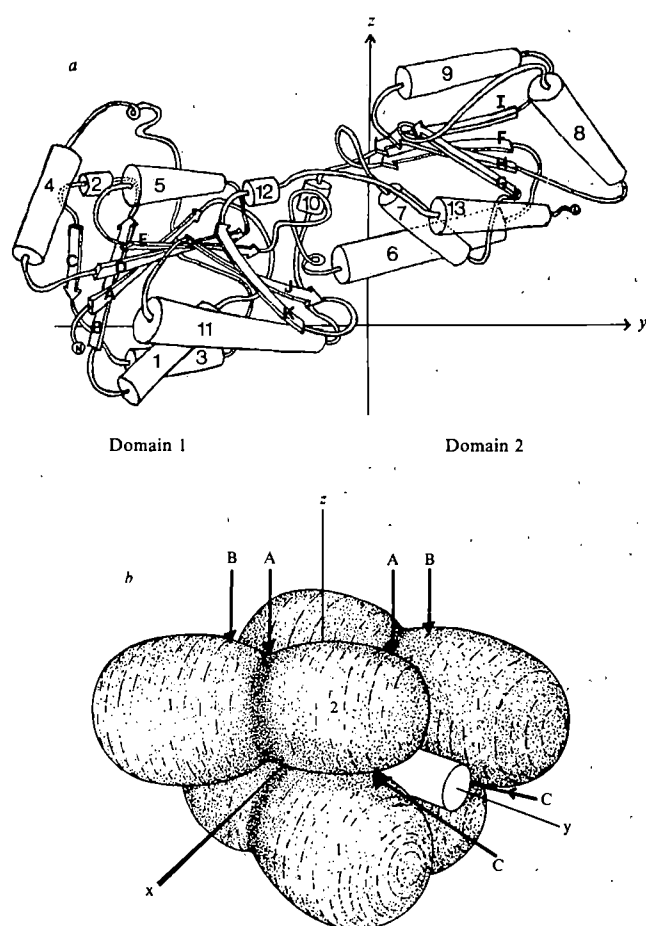
PFK was purified from cell extracts of *B. stearothermophilus* by affinity chromatography on AMP-Sepharose<sup>6</sup> and ATP-agarose<sup>7</sup>. Large crystals suitable for X-ray diffraction were grown from ~2 M potassium phosphate pH 7.3 containing 2 mM fructose-6-phosphate, 1 mM dithiothreitol and 0.5 mM EDTA. X-ray photographs show that the crystals belong to space group I222 with cell dimensions 122.5 Å, 84.1 Å, 61.5 Å and with one subunit in the asymmetric unit. Native crystals were prepared for data collection by washing out the F6P with 2.5 M phosphate solution. Intensity data were collected to 2.4 Å resolution on a rotation camera for the native and three heavy atom derivatives, (*p*-hydroxymercuribenzoate, ethylmercury thiosalicylate, and Au(CN)<sub>2</sub><sup>-</sup>). An electron density map was calculated by the usual method of isomorphous replacement using phases calculated from the three heavy atom derivatives.

A phase improvement method suggested by Agarwal and Isaacs<sup>8</sup> was tried, starting with the isomorphous replacement map. A model structure was created by distributing atoms of atomic number 7 through the high density regions of the map, such that the number of atoms in the asymmetric unit was approximately correct (~2,400) and they were not too close together. The positions and thermal parameters for these dummy atoms were refined by a diagonal least-squares procedure<sup>9,10</sup>, and the calculated structure factors after eight cycles of refinement ( $R = 0.255$ ) were used to calculate phases. The calculated phase probability distributions were combined with those from the isomorphous derivatives<sup>11–13</sup>, and a second electron density map was calculated with these combined phases. Although the theoretical justification for this method is dubious, particularly at a resolution as low as 2.4 Å where the ratio of observations to parameters is only about 1.35, it does seem to sharpen the electron density in a useful way.

The two electron density maps were contoured on to small

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**Fig. 1** *a*, Schematic drawing of the polypeptide chain of one subunit, viewed along the *x* axis. Arrows represent  $\beta$ -sheet strands (A-K) and cylinders represent  $\alpha$ -helices (1-13). *b*, Schematic drawing of the tetramer of PFK. Each subunit is shown divided into two domains, numbered 1 and 2. The four subunits are related by three orthogonal dyad axes (222 symmetry), which coincide with the crystallographic symmetry axes. There is a solvent filled hole of  $\sim 7$  Å diameter through the centre of the tetramer along the *y* axis, indicated by a cylinder. The positions of some of the sites A, B and C are marked, showing that sites A and C lie between subunits.

scale map stacks ( $1 \text{ Å} = 2.5 \text{ mm}$ ). From these we could trace the course of the entire polypeptide chain, and locate nearly all the amino acid side chains. It was easier to trace the main chain through the map from the combined phases, but more recent model building into the density on a computer graphics system<sup>14</sup> has shown that the shape of the electron density is better in the isomorphous map. Nevertheless, the procedure was useful in aiding the initial interpretation and improving some of the poorer regions of the density. Part of the isomorphous replacement map is shown in Fig. 2, showing the top of domain 1 and the central  $\beta$ -sheet of domain 2. The interpretation of nearly all of this map was clear, particularly in the helices and the  $\beta$ -sheet regions. The positions and shapes of the side chains agreed with the chemical sequence, aromatic residues and methionines being unmistakable. In the internal parts of the structure, most of the main chain carbonyl groups could be seen as bulges on the electron density. A few of the loops on the surface were less clear, particularly the loops between helix 1 and sheet strand B, and between helices 3 and 4. Details of the crystallography will be published elsewhere.

### The structure of the protein

This description is based on the approximate  $\alpha$ -carbon coordinates measured from small-scale map at 2.4 Å resolution. The more precise model building into the electron density confirms

all the essential features. Figure 1*a* illustrates one subunit, and Fig. 3 shows the  $\alpha$ -carbon chain in stereo with the subunit interactions, the substrates and effectors. Figures 4 and 5 are also schematic views of two halves of the tetramer.

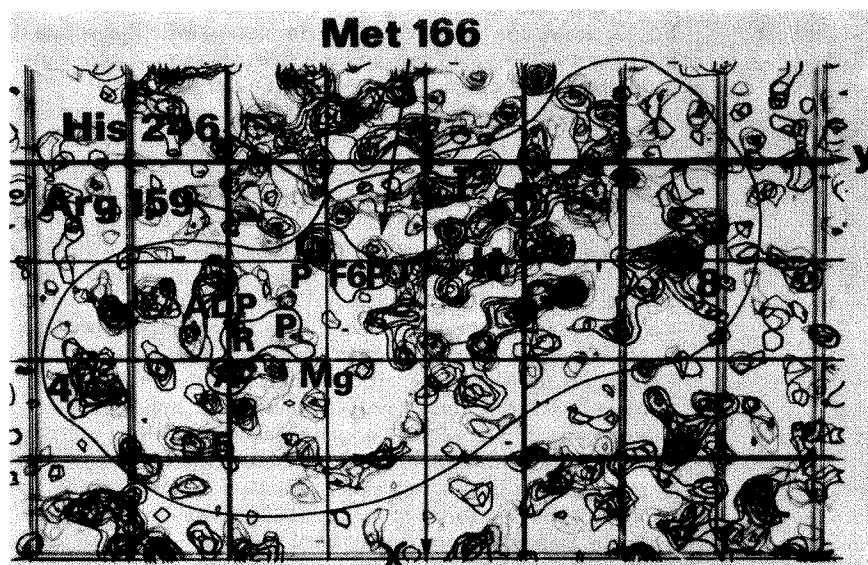
The PFK subunit consists of two domains each of which has a central  $\beta$ -sheet sandwiched between  $\alpha$ -helices, a type of fold which has been seen in a number of proteins ( $\alpha/\beta$  class of proteins of Levitt and Chothia<sup>15</sup>). However, the topology of the  $\beta$ -sheet strand connections in both domains of PFK is different from that in any other known protein structure. The first domain (Fig. 1*a*) has seven strands of  $\beta$ -sheet, the central five being parallel, and the outer two antiparallel to the others. The second domain has four parallel strands. The two sheets point towards a deep cleft which forms the active site (see below). Starting from the amino terminus, the chain first forms the main part of domain 1, including five strands of sheet A to E, and helices 1-5, then crosses to the second domain through the long helix 6. There follows most of domain 2, then the chain returns to domain 1 for the last two sheet strands J and K. Finally an extended chain returns to domain 2 for the final helix 13. Domain 1 consists approximately of residues 1-131 and 251-301, and domain 2 of residues 132-250 and 302-316. This insertion of one domain into the middle of the polypeptide chain of another is unusual in domain proteins: in most cases the domains are segregated along the chain, although the final crossing to the other domain for a C-terminal helix is more common (for example, glyceraldehyde-3-phosphate dehydrogenase<sup>16,17</sup> and phosphoglycerate kinase<sup>18</sup>). Other proteins with such inserted domains include pyruvate kinase<sup>19</sup> and the *E. coli* arabinose-binding protein<sup>20</sup>. All but one of the connections between successive parallel  $\beta$ -strands are right-handed<sup>21,22</sup>: the exception is the connection between strands E and J, where the connecting loop contains the whole of the second domain, about 157 residues.

Each subunit forms close contacts with only two of the other subunits in the tetramer. The interaction across the dyad along *z* is shown in Figs 3*a* and 4: the end of helix 6 is tightly packed against the neighbouring subunit, and the  $\beta$ -strands I interact across the dyad axis. The relative twist of these strands is in the wrong direction for extensive antiparallel  $\beta$ -sheet interactions, but two hydrogen bonds are formed across the dyad axis. The other subunit contact, across the dyad along *x*, is shown in Figs 3*b* and 5. The main interaction is the packing between the three helices 1, 3 and 11, and three helices 6, 7 and 13 in the other subunit. Also the bend between the  $\beta$ -strands J and K is in contact with its counterpart across the dyad. Figure 3*c* is a view of the tetramer from the end of the subunits, along *y*, and shows both of these subunit contacts. A solvent-filled hole  $\sim 7$  Å in diameter passes through the middle of the tetramer separating the pairs of subunits which do not interact.

**Table 1** Compounds binding to the three binding sites A, B and C

A	Active site	Effector site
	B	C
F6P	ADP/Mg or Mn	$P_i$
$P_i$	AMPPNP/Mg or Mn	ADP/Mg or Mn (activator)
(FDP)	(ATP)	PEP (inhibitor)

Compounds in brackets have not been observed, but are assumed to bind in these sites. Binding of ligands other than  $P_i$  in site C is only observed in crystals which have been transferred to 1.25 M sodium citrate (pH 7.3), or to 2.5 M potassium tartrate (pH 7.7). In the citrate solutions, and tartrate solutions containing ADP, the phosphate ion in site C is displaced, but the phosphate in site A remains. Transfer of the crystals to tartrate solution in the absence of other ligands causes the crystals to crack and change their space group, suggesting that a structural change has occurred. No binding of tartrate or citrate ions is apparent.



**Fig. 2** Sections  $z = 20/76$  to  $26/76$  of the isomorphous replacement electron density map. This view corresponds to those in Figs 3a and 4. The grid lines are at intervals of  $10 \text{ \AA}$ . One subunit is outlined: part of the subunit related by the dyad axis marked on the  $z$  axis is shown at the top. Outlines of the difference electron density for F6P and ADP are shown. The F6P outline encloses the high density peak representing the inorganic phosphate ion in the native map (labelled P). The ADP outline is labelled A for adenine, R for ribose, P for the  $\alpha$ -phosphate, and Mg for the magnesium ion. Three of the side-chains which bind the F6P molecule are indicated: His 246 and Arg 159 (from the upper subunit) bind the phosphate together with Arg 240, which is above the sections shown. Met 166 is one of the residues forming the pocket for the fructose ring. The four  $\beta$ -sheet strands of domain 2 are labelled F, G, H, I, and three  $\alpha$ -helices are labelled 4, 5 and 8 (compare Fig. 4).

## Binding studies

The binding of several compounds has been studied in the crystal, mostly using data to  $6 \text{ \AA}$  resolution, but with one set of data to  $2.4 \text{ \AA}$  resolution. There are three binding sites per subunit, sites A and B forming the active site and site C which appears to be the effector site. The molecules which bind to these sites are summarised in Table 1. Site A binds F6P in the crystals as grown, but this is replaced by  $\text{PO}_4^{3-}$  when F6P is washed out to make the native crystals. The phosphate ion is the highest peak in our electron density map (Fig. 2). Presumably this site should also bind fructose-1,6-diphosphate (FDP), but crystals soaked in this compound show only weak binding. Site B binds ADP with either  $\text{Mg}^{2+}$  or  $\text{Mn}^{2+}$ , but difference maps from crystals soaked in ATP show density identical to ADP, perhaps because of hydrolysis of ATP by the enzyme. The ATP analogue AMPPNP (5'-adenylylimidodiphosphate) binds in this site also with either  $\text{Mg}^{2+}$  or  $\text{Mn}^{2+}$ , and shows additional electron density which presumably represents the  $\gamma$ -phosphate group. Site C appears to be the effector site, and binds a phosphate ion in our native crystals: this ion also shows clearly in the electron density map. This site binds both the activator ADP and the inhibitor PEP.

## Details of the binding sites

Figures 4 and 5 show the binding of the substrate pair F6P and ATP/Mg in the active site, and the activator ADP/Mg in the effector site. These are superimposed on diagrams of two subunits from the tetramer, together with those side chains which are in contact with the ligands. The positions of the bound molecules were taken from a difference map at  $2.4 \text{ \AA}$  resolution from crystals soaked in F6P, ADP and Mg in  $2.5 \text{ M}$  potassium tartrate. This map showed clear density for F6P in site A and ADP/Mg in the effector site C, but the ADP/Mg in site B was less clear and only about half occupied: there was no electron density for the  $\beta$ -phosphate. Models of the three ligands were fitted to the electron density on a small Richards' box. The drawings show ATP in the active site, based on the  $2.4 \text{ \AA}$  resolution ADP density and the  $\gamma$ -phosphate position from a difference map between AMPPNP and ADP at  $6 \text{ \AA}$  resolution. The magnesium ions are clear in the difference electron density and correspond to their positions deduced from the  $6 \text{ \AA}$  resolution difference map between  $\text{Mn}^{2+}$  and  $\text{Mg}^{2+}$ .

**Site A.** The notable feature of this site is that the 6-phosphate group of F6P (and the phosphate ion in the native crystals) is

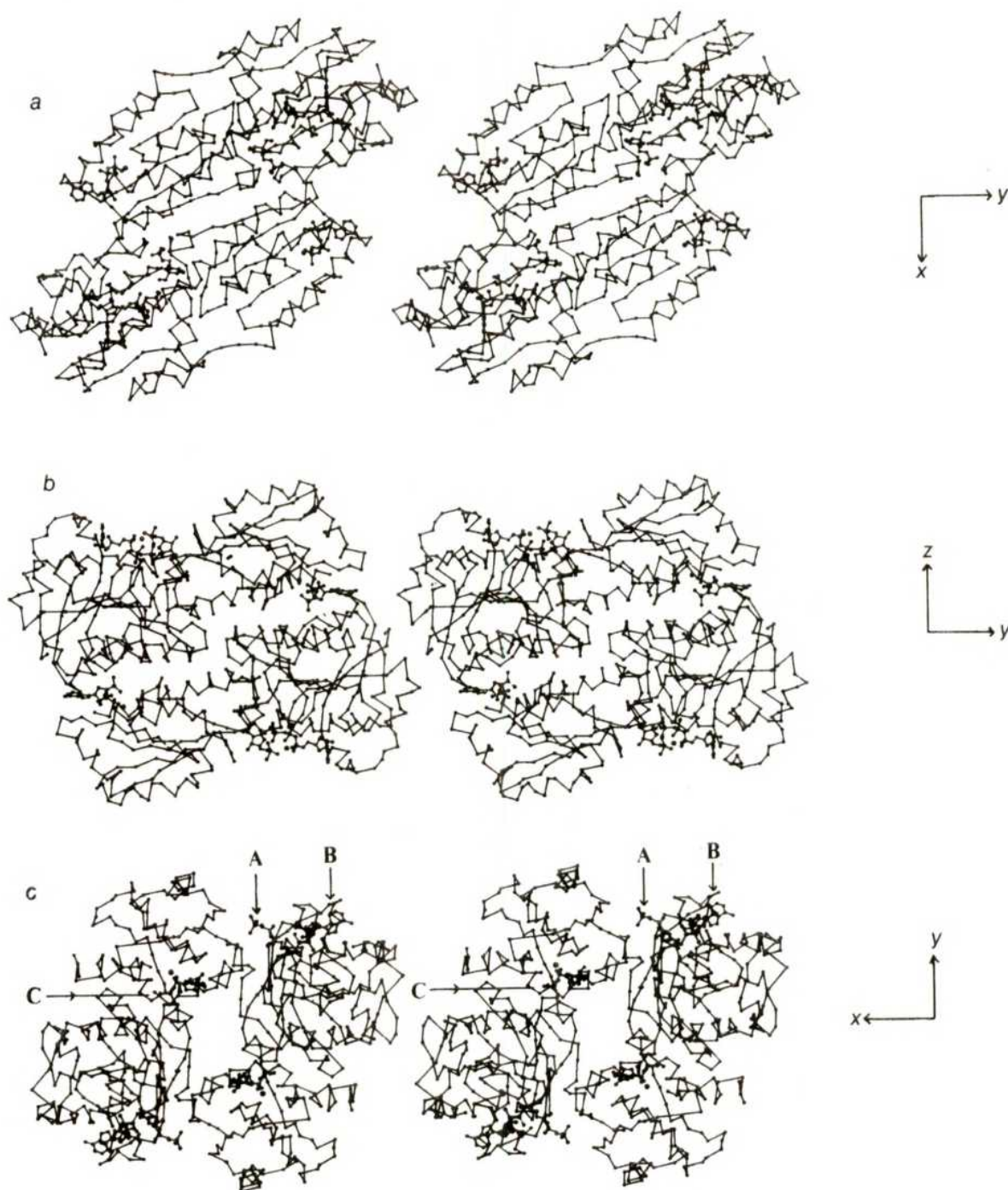
bound by amino acid side chains from two adjacent subunits, His 246 from one subunit, and Arg 159 and Arg 240 from the other subunit (Fig. 4). Thus the cooperative ligand F6P bridges between two subunits. The 1-hydroxyl is close to the  $\gamma$ -phosphate of ATP, and appears to be hydrogen bonded to Asp 124. This residue may act as a base catalyst for the reaction by increasing the nucleophilicity of the  $1\text{-OH}$  group. This phosphorylation site lies in the boundary between the two domains of the subunit, while the groups which bind the sugar ring of F6P (Arg 249, Met 166, Glu 219 and Asp 124) come from domain 2 of the subunit (the right-hand domain in the lower subunit in Fig. 4).

**Site B.** The conformation of ADP (and hence ATP) in this site is not very clear from our current maps, nor are all the interactions with side chains. The magnesium ion binds in the same position with both ADP and AMPPNP (and therefore presumably with ATP). In ADP it binds to the  $\alpha$ -phosphate and possibly to the invisible  $\beta$ -phosphate: in ATP it probably bridges the  $\alpha$ - and  $\beta$ -phosphate groups. The ATP molecule is bound to domain 1 with the  $\gamma$ -phosphate near the domain boundary: the only residue involved from domain 2 is Arg 168, which becomes ordered near the phosphates when the nucleotide is bound. Asp 100 may be involved in the binding of  $\text{Mg}^{2+}$ .

**Site C.** While the substrate F6P is bound between the subunits related by the  $z$  dyad axis, the effector is bound in a cleft between subunits related by the  $x$  dyad. The key interaction with ADP seems to be with the  $\beta$ -phosphate group, which replaces the phosphate ion bound to this site in the native crystals. This phosphate is bound by three arginine residues, Arg 151 from the upper subunit in Fig. 5, and Arg 21 and Arg 25 from the lower subunit. The ribose is bound by His 212 and Thr 155. The  $\text{Mg}^{2+}$  ion bridges the  $\alpha$ - and  $\beta$ -phosphates of ADP, but its only interaction with the protein seems to be with a main chain carbonyl group. The diphosphate of ADP points into a cleft, which is consistent with the specificity of the site for ADP rather than AMP or ATP, and its lack of specificity for the base<sup>5</sup>. The phosphate group of PEP appears to bind in the same place as the  $\beta$ -phosphate of ADP. This site is best considered as a phosphate binding site, rather than a nucleotide binding site.

## Discussion

This structure of *B. stearothermophilus* PFK seems to correspond to the active R-state of the Monod, Wyman, Changeux model<sup>5,23</sup>. A structural change caused by transferring the crystals to tartrate solution suggests that a second conformational state exists. All the structures we have been able to look at contain either phosphate ion or F6P bound in site A, and we



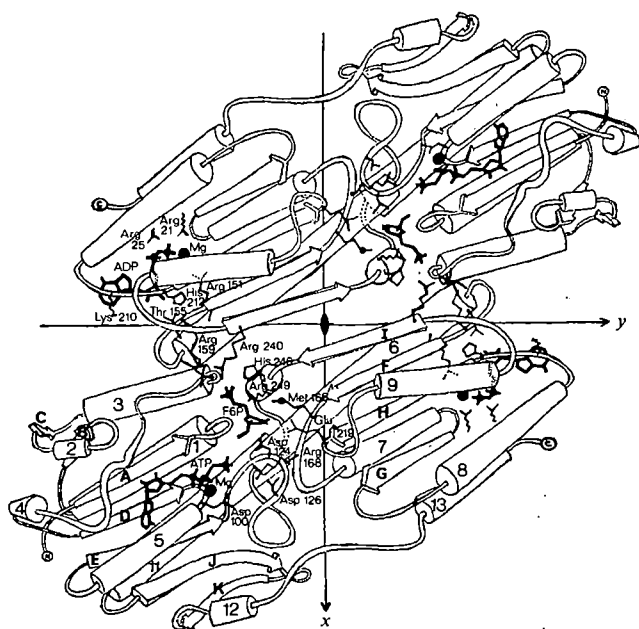
**Fig. 3** Orthogonal stereo views of the  $\alpha$ -carbon atoms of PFK together with the substrates F6P and ATP/Mg, and the activator ADP/Mg, viewed towards the centre of the tetramer. *a*, The upper two subunits of the tetramer viewed along the *z* axis (compare Fig. 4). *b*, The upper two subunits viewed along the *x* axis (compare Fig. 5). *c*, The upper half of the tetramer viewed along the *y* axis. Only half of each subunit is shown. The three ligand binding sites are indicated.

suggest that the removal of these ligands triggers the structural change to the less active T-state. Without knowing its structure, we cannot account in detail for the difference in affinity for ligands between the two states, but the nature of the binding site suggests a plausible explanation. The F6P site bridges two subunits, so a change of the relative position of these subunits could remove the two arginines 159 and 240 from the F6P site, reducing its affinity for F6P without changing the mode of binding. As the active site of PFK lies between the two domains of the structure, it would seem likely that any relative movement of these domains would change the catalytic rate,  $k_{cat}$ , by moving

the catalytic groups. As a change of  $k_{cat}$  is not observed, a more plausible model for the allosteric transition is a rearrangement of essentially rigid subunits into a different quaternary structure. This would contrast with other enzymes in which two domains within a subunit move on binding of substrates: these include hexokinase<sup>24</sup>, glyceraldehyde-3-phosphate dehydrogenase (A. J. Wonacott, personal communication) and probably phosphoglycerate kinase<sup>18</sup>.

The effector site lies at the interface between another pair of subunits; as it binds both activator and inhibitor, the affinity for these molecules must change in opposite senses during the





**Fig. 4** Schematic view of the two subunits along the *z* axis towards the centre of the tetramer. The substrates and activator are shown, and the side chains around the active site and effector site.

allosteric transition. The local changes around this site are different with binding of ADP and of PEP, but we cannot explain the relative affinities of the two conformations without knowing the structure of the T-state.

Each subunit in the tetramer has an interface with two other subunits, one of these is bridged by F6P, the other by the effector (see Fig. 3c): thus catalysis and control require the whole tetramer. Figure 4 shows that the active site is close to the effector site, and the part of the enzyme that lies between the sites, particularly residues 151 to 159, has side chains pointing in one direction to the F6P site and in the opposite direction to the effector site. Thus binding of a ligand in either site could affect

directly the ligand affinity in the other site, in addition to the indirect effect caused by shifting of the equilibrium between the two conformational states.

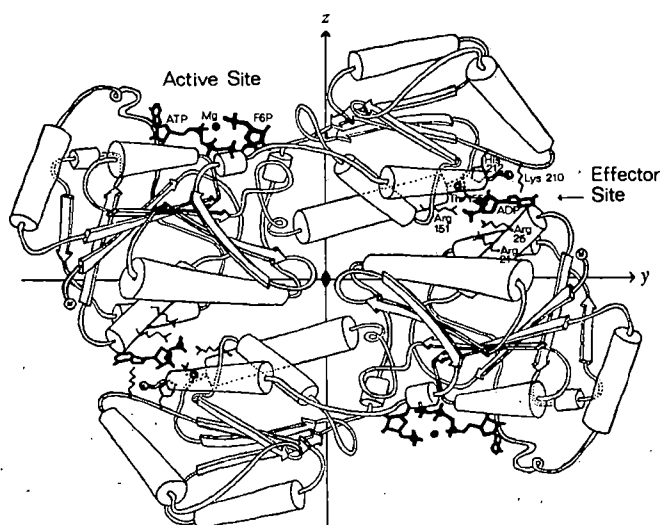
All three binding sites in PFK bind phosphate groups, but there are interesting differences between them. Sites A and C both bind an inorganic phosphate ion as well as their specific ligands, and they have a clear electrostatic mode of binding: site C binds the phosphate with three arginine residues, and Site A with two arginines and a histidine. In contrast, site B does not bind inorganic phosphate, and does not have such a clear positively charged nature, although arginine 168 is close and the  $Mg^{2+}$  ion is bound to the  $\alpha$ - and  $\beta$ -phosphates of ADP and ATP. The phosphates in this site are at the amino end of helix 5, and it has been suggested that the dipole of an  $\alpha$ -helix provides approximately half a positive charge at its amino end<sup>25</sup>. The difference between the three sites may be related to the requirements for binding at sites A and C, compared to binding and catalysis at site B. Site C is unusual in that it lies at the amino end of a  $\beta$ -sheet. In most proteins with parallel  $\beta$ -sheets, the binding sites for all types of ligand are at the carboxy end of the sheet (although the secondary ADP site in pyruvate kinase is at the amino end of the  $\beta$ -sheet barrel<sup>19</sup>). However, this effector site C is required by its function to be in the subunit interface, and this requirement presumably outweighs other preferences.

Over the past few years, there has been much discussion about the similarities between the tertiary structures of some proteins and whether these relationships are due to the rules of polypeptide folding or to evolutionary homology<sup>26-29</sup>. Among proteins with central  $\beta$ -sheets ( $\alpha/\beta$  proteins), four different dehydrogenases resemble each other in one of their two domains. In contrast, five different kinases (adenylate kinase<sup>30</sup>, hexokinase<sup>31</sup>, phosphoglycerate kinase<sup>18,32-34</sup>, pyruvate kinase<sup>19</sup> and PFK) show much less similarity. Although they all contain domains built around parallel  $\beta$ -sheet, the topological arrangement of these sheets are different, and their mode of binding substrates is different. These differences suggest that these kinases do not share a common ancestor.

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**Fig. 5** Schematic view of two subunits along the *x* axis towards the centre of the tetramer. The substrates and activator are shown, and the relevant side chains around the effector site.



# letters

## SS433—a massive black hole?

THE peculiar emission line object SS433 has recently been the subject of interest following new spectrophotometric observations by Margon *et al.*<sup>1</sup>, and previous suggestions identifying it with the supernova remnant (SNR) W50 (ref. 2) and also with the X-ray source A1909+04 (ref. 3). Margon *et al.*<sup>1</sup> report "three very strong (equivalent widths 50–150 Å) broad emission features in the green, red and IR, which change in intensity, profile and wavelength daily." In this note we propose that the reported red and IR lines (at  $\sim 6,000$  Å and  $\sim 7,400$  Å, respectively) are Doppler-shifted H $\alpha$  lines which are emitted from a ring of matter orbiting around a  $\sim 10^6 M_\odot$  black hole. (The green line at  $\sim 5,200$  Å may be the redshifted H $\beta$  line.) The ring itself may be part of an accretion disk and its radius is  $\sim 20$ – $25$  gravitational radii ( $R_g = 2GM/c^2$ ). The intensity of an emission line per observed Doppler-shifted frequency interval in such a ring is maximal at the two extreme ends of the spectrum<sup>4</sup>, in any case of emission constant along the ring. These maxima are quite sharp; in the non-relativistic case, we have

$$dI_\nu/d\nu \propto \{\nu_0^2 - (\partial\nu)^2\}^{-1/2}$$

where  $\partial\nu$  is the frequency shift and  $\nu_0$  depends on the radius of the ring and on its inclination with respect to us. Also, due to the gravitational redshift in our case, the centre-of-mass of the red and IR lines emitted from such ring is additionally redshifted.

It is difficult to determine the relative intensity of the Doppler-pair members observationally, because of the uncertainty in the reddening out to SS433; if one takes  $A_V \sim 7$  mag, as is reasonable for a distance of  $\sim 3.3$  kpc in the galactic plane, the data of Margon *et al.* are consistent with both members having roughly equal intensities or even having the blueshifted member slightly stronger, as expected for such a model<sup>4</sup>. Also, to match these observed shifts<sup>1</sup>, the plane of the ring should be inclined towards us<sup>4</sup>. Our numerical calculations for a model line emitted from such a ring are consistent with the structure of the observed pair in Fig. 3 of ref. 1 for inclination angles of  $45$ – $60^\circ$ .

Accumulated results of observations (J. Katz, personal communication) of the above and other shifted lines from SS433 show the following characteristics:

- (1) Let  $B = (\lambda_{\text{blueshifted}} - \lambda_0)/\lambda_0$ ,  $R = (\lambda_{\text{redshifted}} - \lambda_0)/\lambda_0$  and let  $c$  be the velocity of light. then,  $\frac{1}{2}c(R+B)$  has now varied between  $\sim 10,000$  km s<sup>-1</sup> and  $\sim 40,000$  km s<sup>-1</sup>. These high velocities strongly point to processes in a region of high gravitational field, near a neutron star or black hole.
- (2) During these variations of  $B$  and  $R$ , the centre-of-mass (COM) of the lines ( $\frac{1}{2}c(R+B)$ ) remains fairly constant<sup>5</sup>, redshifted at  $\sim 10,000$  km s<sup>-1</sup>. This poses severe difficulties on models in which the changes in  $R$  and  $B$  are due to velocity magnitude changes alone (rather than to changes in angle, such as a precession of the ring<sup>6</sup>, say, which is suggested by a possible periodicity in the data<sup>5,7</sup>). It also indicates that the emission region for the shifted lines remains at a fairly constant gravitational redshift (that is, distance from the compact object).
- (3) The shifted lines are accompanied by an unshifted line. This poses a difficulty on any interpretation of the shifted COM as an overall motion of SS433.
- (4) The shifted lines are quite narrow,  $(\delta\lambda/\lambda)/B_{\text{max}} \leq 10\%$ , suggesting a reasonably narrow emission region.

(5) Let the line-emitting region be of surface area  $4\pi R^2$ . Then its brightness temperature  $T_b$  must satisfy  $T_b > 10^9$  K  $(R/10^9 \text{ cm})^{-2}$ . This poses severe difficulties on neutron star models and on models having black holes with  $R_g \leq 10^{11}$  cm ( $M \leq 3 \times 10^5 M_\odot$ ), particularly with no observational evidence for coherent emission.

(6) In several of the H $\alpha$  Doppler-shifted pairs<sup>1</sup>, one line seems to be the mirror image of the other (imaging on the  $\lambda$  axis, mirror located at the COM). This indicates a correlation between the geometries of the two emission regions, such as that found in emission from a ring. In pairs observed at other times, no such symmetry was found (J. S. Gallagher, personal communication), suggesting that the emission region has variable structure, or that its appearance to us is variable.

We infer from these considerations that the compact object, around which the emission takes place, cannot be a neutron star or a light black hole, but rather the above mentioned  $\sim 10^6 M_\odot$  black hole. An optically thick accretion disk around such a black hole has indeed most of its flux coming from such ring, at UV and visible frequencies<sup>8</sup>. Note, that if the centre of the SNR W50 is at distance  $\geq 10$  pc from SS433 (ref. 1), the disk may be fed by the SNR (although interstellar matter, if its density near the black hole is close to its average value in the Galaxy, can do the same); for typical values<sup>9</sup> of the particle density ( $\sim 1$  cm<sup>-3</sup>) and velocity ( $\sim 100$  km s<sup>-1</sup>) in such SNR, the feeding is at a rate of  $\leq 10^{-7} M_\odot$  per yr, giving a total luminosity of  $\leq 10^{39}$  erg s<sup>-1</sup> if the disk is in steady state. This is consistent with the observed optical luminosity, which seems to indicate that  $M_v \leq -3.5$  (ref. 1).

The unshifted H $\alpha$  line<sup>1</sup> could arise from reprocessing of some of the  $\leq 10^{39}$  erg s<sup>-1</sup> optical and UV radiation from the black hole, in the rim of the disk<sup>10,11</sup>. This suggests that SS433 might be a strong UV source and that the shifted lines may also be due to reprocessed UV radiation.

Our model also suggests that the whole system may be part of an old globular cluster.

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## Hard X-ray spectrum of Cyg X-1

THE three most likely black hole candidates known in X-ray astronomy are Cyg X-1, Circ X-1 and V 861 Scorpii<sup>1</sup>. Cyg X-1 has a key role as it is the best studied of these objects. Unfortunately, the evidence for its being a black hole is entirely circumstantial; namely, the mass of the compact object and its observed short time variability. The spectrum of Cyg X-1 is also very different from those of accreting neutron stars, but it is similar to spectra of extragalactic sources which may contain massive black holes. Eardley *et al.*<sup>2</sup> have recently reviewed the existing evidence. Here we consider only the X-ray spectrum and the source luminosity. This work was motivated by the spectral measurements with very high statistical accuracy of Cyg X-1 made in the hard X-ray range by the AIT/MPI group<sup>3</sup>. Progress has been made in Moscow in calculating source spectra expected from hot accretion disks. Thus, there is a new basis for a comparison between observational results and theoretical predictions.

Measurements by numerous rocket, satellite and balloon experiments have shown that the Cyg X-1 spectrum is variable on different time scales (see ref. 4). In particular, the source exhibits 'flares' and transitions between high and low spectral states<sup>5,6</sup>. Matteson *et al.*<sup>7</sup> stressed that despite this variability most of the observed spectra lie near a 'normal' spectrum which can be characterised by a power law.

$$\frac{dN}{dE} = 3 \times 10^{-2} \left( \frac{E}{10 \text{ keV}} \right)^{-1.6} \quad (\text{photons cm}^{-2} \text{ s}^{-1} \text{ keV}^{-1}) \quad (1)$$

for  $2 \text{ keV} \leq E \leq E_b$ , while beyond  $E_b$  the spectrum steepens significantly. A range of values has been quoted for this break energy:  $E_b = 32 \text{ keV}$  (ref. 8),  $85 \text{ keV}$  (ref. 7),  $115 \text{ keV}$  (ref. 9) and  $\geq 150 \text{ keV}$  (ref. 10). During the past 10 yr the source has apparently spent most of its time ( $\sim 90\%$ ) in this normal spectral state which has also been called the 'low' or 'non-flaring' state. The 'high' state in which the source is seen temporarily<sup>5</sup> is characterised by a steep spectrum in the 2–10 keV range. As far as the break energy of the normal spectrum is concerned, it is not clear to what extent the quoted differences are due to the difficulty in positioning a 'spectral break' in the presence of statistical and systematic errors. On the other hand, the break energy is a very important parameter, as in the current comptonisation models it is related to the electron temperature of the scattering electrons (see ref. 2).

The hard X-ray spectrum of Cyg X-1 has been measured with high accuracy by the balloon group of the Astronomisches Institut Tübingen and MPI Garching on three different flights: 20 February 1975; 2 May 1976 and 20 September 1977. These measurements cover the spectral range from 15 to 150 keV. Figure 1 shows the data obtained on 20 September 1977 which have unprecedented accuracy.

The spectra taken during these three flights agree with each other to within  $\sim 30\%$  and lie very close to the normal spectrum quoted above. They all show a steepening below 100 keV (ref. 3). Furthermore, they agree both in spectral slope and absolute intensities (within  $\sim 40\%$ ) with high quality rocket spectra taken by the GSFC group on 4 October 1975<sup>11</sup>. All these data suggest that the normal state has a more definite intensity (to  $\leq 40\%$ ) than previously assumed.

The X-ray emission of Cyg X-1 has been explained in terms of disk accretion on to a black hole<sup>12–14</sup> which is determined by the following parameters<sup>13</sup>: (1) the mass of the black hole  $m = M_x/M_\odot$ ; (2) the accretion rate  $\dot{m} = \dot{M}/\dot{M}_{\text{edd}}$  where  $\dot{M}_{\text{edd}}$  is the Eddington critical accretion rate; (3) the turbulence parameter  $\alpha$ .

At small accretion rates ( $\dot{m} \ll 1$ ) the disk is stable but cool and unable to produce the observed hard X-ray spectrum. If the

accretion rate (onto a Schwarzschild black hole) becomes

$$\dot{m} \geq \frac{1}{50} (\alpha m)^{-1/8} \quad (2)$$

the radiation pressure will exceed the gas pressure in the inner part of the disk<sup>13</sup> which leads to both secular<sup>15</sup> and thermal<sup>16,17</sup> instabilities. The unstable inner regions consist of hot and cold zones in pressure equilibrium. This means that the cold zones are dense and the production of low energy photons by bremsstrahlung and other collisional mechanisms is very effective. At the same time, these mechanisms are rather ineffective in the hot zones and hard X rays are mainly produced by comptonisation of the soft photons in the hot zone of the disk. This comptonisation is also the main cooling mechanism for the hot plasma.

As far as the physical nature and the detailed configuration of the hot gas component is concerned, various models have been discussed: a hot inner disk surrounded by a cooler disk<sup>18,19</sup>, instability driven random outbursts of hot gas<sup>16</sup>, a hot corona surrounding a cool disk<sup>20–22</sup>. The comptonisation of low energy photons in the high temperature electron clouds leads to the formation of power law X-ray spectra

$$J(\nu) \sim \nu^{-n} \quad \text{for } h\nu < kT_e \quad (3)$$

while above  $kT_e$  the spectrum cuts off roughly exponentially.

This was first shown in numerical calculations by Katz<sup>23</sup> for a non-relativistic plasma and by Pozdnyakov *et al.*<sup>24</sup> for relativistic and semi-relativistic plasmas using Monte Carlo methods. Shapiro *et al.*<sup>25</sup> found an approximate analytical formula for the spectral index of the power law spectrum.

Recently, Sunyaev and Titarchuk<sup>26</sup> derived an analytical solution for the comptonisation spectrum from a spherical, isothermal cloud in which a source of soft photons is embedded. This solution is valid for any spectral shape if the injected soft photons have an energy  $h\nu_{\text{soft}} \ll kT_e$ . The resulting high energy spectrum only weakly depends on the geometry of the clouds. For sufficiently high energies ( $h\nu \gg kT_{\text{soft}}$ ), the analytical solution for the comptonisation spectrum can be written using the integral representation of a Whittaker function

$$J(E) = Ax^3 e^{-x} \int_0^\infty t^{n-1} e^{\left(1 + \frac{t}{x}\right)^{n+3}} dt \quad (4)$$

where  $A$  is a constant and

$$\left. \begin{aligned} x &= \frac{E}{kT_e} = \frac{h\nu}{kT_e} \\ n &= -\frac{3}{2} + \left(\frac{9}{4} + \gamma\right)^{1/2} \\ \gamma &= \frac{\pi^2}{3} \frac{m_e c^2}{kT_e(\tau_0 + \frac{2}{3})^2} \end{aligned} \right\} \quad (5)$$

$\tau_0$  is the Thomson optical thickness of the electron cloud. In the low frequency limit  $x \ll 1$ , one easily obtains from the integral representation of the Whittaker function that the spectrum is a power law (the same as obtained by Shapiro *et al.*<sup>25</sup>):

$$J(E) \sim x^{-n} \quad (6)$$

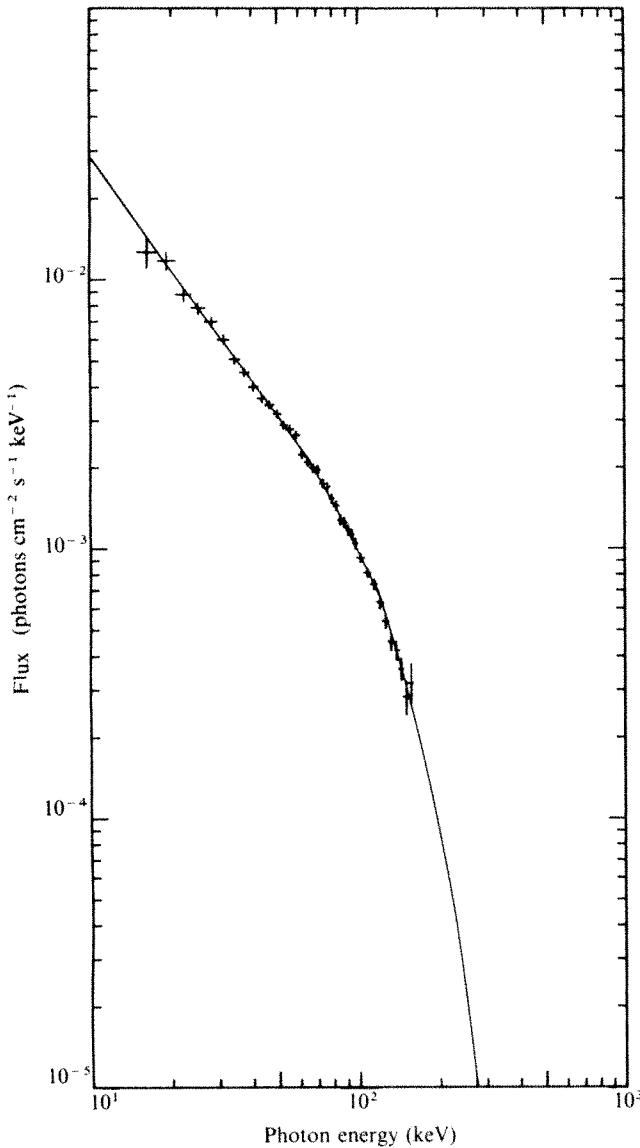
Likewise in the high frequency limit  $x \gg 1$  the spectrum is of the Wien-type

$$J(E) \sim x^3 e^{-x} \quad (7)$$

A fit of the spectrum given by equation (4) to the observed low state spectrum (Fig. 1) yields the following set of parameters:

$$\begin{aligned} kT_e &= 27 \text{ keV} \\ \tau_0 &= 5 \end{aligned} \quad (8)$$

Figure 1 shows the theoretical curve which is an excellent fit to



**Fig. 1** Low state spectrum of Cyg X-1, observed on 20 September 1977 by the MPI/AIT balloon experiment<sup>3</sup>. The solid curve represents a photon spectrum expected by comptonisation of soft photons in an electron cloud with a temperature of  $2.5 \times 10^8$  K and a Thomson optical thickness  $\tau_0 = 5$ .

the experimental data. Note that the temperature ( $T_e \sim 3 \times 10^8$  K) is much less than that obtained in previous papers<sup>2,25</sup> ( $T \sim 10^9$  K).

We conclude that the comptonisation of soft photons by a hot gas gives a very good description of the observed low state spectrum. As the hard X-ray band (30–100 keV) contains the main part of the overall luminosity, this radiation must come from the inner region of the disk where the main energy release occurs. This region is quite compact (see ref. 13) and the larger and cooler outer regions do not contribute appreciably to the low state flux.

An integration of the spectrum for  $E \geq 1$  keV yields

$$L_x = 2.3 \times 10^{37} \left( \frac{d}{2.5 \text{ kpc}} \right)^2 \text{ erg s}^{-1} \quad (9)$$

Thus, for a distance of 2.5 kpc (ref. 27) the observed luminosity is roughly 65 times below the Eddington critical luminosity for a  $10 M_\odot$  black hole which is given by

$$L_{\text{edd}} = 1.3 \times 10^{39} \left( \frac{M_x}{10 M_\odot} \right) \text{ erg s}^{-1} \quad (10)$$

Therefore, the accretion rate  $\dot{m}$  derived from observations is a factor  $\sim 1.3$  lower than that required to obtain an unstable disk according to equation (2).

Before discussing this result we shall summarise briefly the requirements on the soft X-ray source feeding the comptonisation process. According to Sunyaev and Titarchuk<sup>26</sup>

$$L_x = L_{\text{soft}} \left( 1 + 2.9 \frac{kT_e}{\langle h\nu_{\text{soft}} \rangle} \right)^{1-n} \quad (11)$$

which for  $L_x = 2.3 \times 10^{37} \text{ erg s}^{-1}$ ,  $kT_e = 27 \text{ keV}$  and  $n = 1.56$  yields

$$L_{\text{soft}} \approx 10^{36} \left( \frac{100 \text{ eV}}{\langle h\nu_{\text{soft}} \rangle} \right)^{0.44} \text{ erg s}^{-1} \quad (12)$$

What is the black-body temperature of the region where the main energy release occurs? For a Schwarzschild black hole this region has a radius of  $\sim 7$  gravitational radii ( $r \sim 2 \times 10^7 \text{ cm}$ ). The total luminosity of this region is given by  $2\pi r^2 \sigma T_{\text{soft}}^4$ . Equating this to  $L_{\text{soft}}$  as given by equation (12) and using

$$kT_{\text{soft}} = \frac{\langle h\nu_{\text{soft}} \rangle}{2.7}$$

we find

$$kT_{\text{soft}} \approx 100 \text{ eV} \quad (13)$$

The soft X-ray source must possess a luminosity of a few times  $10^{35} \text{ erg s}^{-1}$  and a temperature of the order of  $10^6 \text{ K}$ .

We now reconsider why the observed X-ray luminosity is not sufficient (by a factor 1.3) to make the disk unstable. Within this framework of the black hole disk accretion models there are two solutions: (1) the discrepancy of a factor of  $\sim 1.3$  is due to uncertainties in the observational and theoretical figures; and (2) there could be a large contribution to the radiation pressure due to unobservable (soft X and XUV) photons.

In the first case, the accretion disk must be quite close to the lower boundary (in  $L_x$ ) of the region of instability. This is true if Cyg X-1 is a  $10 M_\odot$  black hole, but also if it is a Kerr black hole for which the requirements on the mass accretion rate and total luminosity would be a little lower than given by condition (2). The marginal instability may lead to the total disappearance of the hard X-ray flux, if the mass accretion rate drops below the critical value. Although this critical mass accretion rate is not known precisely (it may be below the 'normal' rate by a factor of 2 to 3) it should represent a sharp threshold which is different for Schwarzschild and Kerr black holes. To our knowledge, there is no observational evidence for such 'off-states'. However, they might be very short (several seconds) if they are due to stochastic fluctuations of the accretion rate in the central parts of the disk. The detection of such short 'off-states' in the X-ray flux from Cyg X-1 and the other black hole candidates would be very important. In the long run, a precise determination of the threshold X-ray luminosity might yield information about the metric of the accreting black hole.

The other possibility is that the actual accretion rate and luminosity are considerably higher than the observed values, so that the disk is permanently unstable. This contribution to the overall luminosity must be substantial ( $\geq 2 \times 10^{37} \text{ erg s}^{-1}$ ) and should consist of soft X-ray and XUV photons. The corresponding sources may be the cool parts of the disk, however, the required luminosity is much larger than that given by equation (12). Therefore, this case requires that the bulk of the soft flux freely escapes from the disk while only a small part (a few per cent) is subjected to the comptonisation process. A condition for this is, of course, that the hot gas clouds cover only part of the disk. Such large 'soft' luminosity of the disk would require a higher temperature ( $kT_{\text{soft}} \sim 250 \text{ eV}$ ) than given by equation (13). Soft X-ray observations with high sensitivity may indicate a soft component in the low state spectra. Optical observations of the accretion disk would be important in this context as they may give information about the total energy flux which heats the peripheral parts of the disk<sup>28</sup>.

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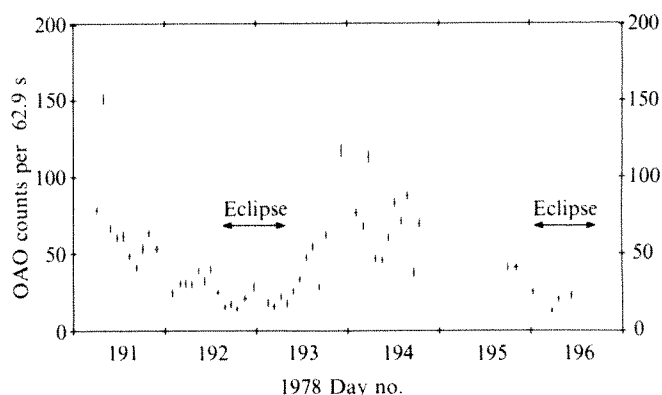
## Search for short time-scale periodicity in the X-ray flux of 4U1700-37

THE first suggestion<sup>1,2</sup> of the existence of a  $\sim 24$  min periodicity in the X-ray light curve of 4U1700-37 was followed by the report<sup>3</sup> of the detection of a  $\sim 97$ -min period in the X-ray data from the SAS C satellite when pointing at the same source. We report here how a new recent Copernicus observation of 4U1700-37 was used to test the existence of a short-term modulation of the X-ray flux.

In July 1978 the Copernicus satellite observed 4U1700-37 for a complete binary cycle between day no. 191.2 and day no. 194.8 (from phase 0.48 of a cycle to phase 0.52 of the following, according to the ephemeris given in ref. 2) and again between day no. 195.7 and day no. 196.5 (phase 0.80-0.01). The data collecting time of the X-ray instrumentation<sup>4,5</sup> was 62.9 s every 86.5 s (23.6 s dead time). The X-ray data are shown in Fig. 1, integrated in bins of  $\sim 1$  h duration, after subtraction of the diffuse sky and particle background. A low amount of contamination ( $\sim 20$  counts per 62.9 s on average) from the nearby X-ray source Sco X-2 is present and visible during the 4U1700-37 eclipses. This would not affect the detection of a periodic modulation, if any, in the X-ray flux of 4U1700-37, but it does increase the noise in the search for periodicities.

For our analysis we used all the July 1978 Copernicus X-ray data relative to the non-eclipsed part of 4U1700-37 light curve. The data sampling interval of 86.5 s allowed the search

for periodicities down to  $\sim 3$  min (corresponding to the Nyquist frequency). Because of the Earth occultation and the regions of high particle background where the detector was switched off the data sets are not regularly spaced, so we used the technique of Gray and Desikachary<sup>6</sup> to Fourier analyse the data. We prewhitened the data of the low-frequency modulations by subtracting a low-order polynomial and we computed the periodograms of the residuals. No evidence of periodicity is present either in the range 20-30 min or 90-100 min, as well as over all the range down to  $\sim 3$  min. Most of the low-frequency noise is introduced in the periodograms by the intense X-ray flaring activity of 4U1700-37. This is the main problem to be overcome when searching for periodic modulation in the flux of a very variable source. We take the maximum value of the random noise peak-to-mean modulation as the upper limit to any true periodicity. Table 1 lists such upper limits for the ranges of period of interest and for increasing order of the polynomial



**Fig. 1** The X-ray light curve of 4U1700-37, after subtraction of the diffuse sky and particle background, as observed by Copernicus in July 1978. The crosses represent the  $1\sigma$  errors on the (3.1-9.4 keV) count-rate integrated in bins of  $\sim 1$  h duration. The contamination of Sco X-2 is low ( $\sim 20$  counts per 62.9 s on average) due to the favourable orientation of the detector's field of view (the efficiency at the location of Sco X-2 was  $\sim 20\%$ ). The times of phase 0.0 (centre of the X-ray eclipse) were calculated from the ephemeris given in ref. 2; the indicated length of the eclipse corresponds to the shortest ever observed (1975, Copernicus; ref. 2.). The gap in the data during day no. 195 is due to the observation of a different target.

fitted to the data. This was an attempt to decrease the low-frequency noise contribution to the periodograms so as to make a real, if any, periodicity show up. Table 1 shows that the technique is most useful to reduce the noise in the range of longer periods investigated. We have also searched for the suggested periodic behaviour in the 4U1700-37 X-ray light curve by folding the data modules' values in the ranges 23-25 and 96-98 min (at intervals of 0.1 min). The analysis is made difficult again by the intrinsic variability of the flux (the average peak-to-mean modulation is  $\sim 20\%$  at any value of the trial period, in agreement with the results of the Fourier analysis). No significant periodicity is evident above the noise.

The fact that the most recent X-ray observation of 4U1700-37 does not show evidence of any of the proposed periodicities can be interpreted in several ways.

(1) The true period is at  $\sim 24$  min, but it has already been shown<sup>2</sup> to be present at times and absent at others (possibly covered by the flaring activity of the X-ray source). It is, therefore, quite possible that it was again absent in the 1978 Copernicus observation. In this respect it is interesting that the mean X-ray flux of 4U1700-37 may have monotonically decreased during 1974-76 (see Table 2 in ref. 2). In the 1978 Copernicus data the X-ray flux is close to the value observed in 1975, also when no periodic modulation was detected.



**Table 1** Upper limits to the peak-to-mean amplitude of any periodic modulation in the ranges of period indicated

Order of the polynomial used to prewhiten the data	Ranges of period (min)	
	20–30	90–100
1	18%	50%
3	19%	17%
6	17%	19%

(2) If a periodicity at  $\sim 97$  min existed in March 1977<sup>3</sup> and was rotational in origin, it is possible that it could have disappeared in the 15 months which elapsed between the 1977 SAS C and the 1978 Copernicus observations (see the current theories of accretion torques on neutron stars in binary systems<sup>7,8</sup>). However, the rotation mechanism seems unlikely because of the very small probability of observing such a rapid changing period with the limited coverage of the satellites available for X-ray astronomy (the periodicity would only be present for a very short interval in the lifetime of the X-ray source). If a different origin for the proposed  $\sim 97$  min periodicity is invoked, then it is difficult to explain how such a strong modulation (up to 60% (ref. 3)) of the X-ray flux has escaped detection in all the other Copernicus observations of 4U1700–37 (one binary cycle in July 1974 (ref. 9), two cycles in July 1975 and August 1976 (ref. 2), one cycle in July 1978). In this context, it has been suggested<sup>10</sup> that the  $\sim 97$  min period in the SAS C data could be spurious, arising from the way the data were collected, namely gaps in the data (due to the satellite being in the South Atlantic Anomaly or Earth blocked, or to telemetry dropouts) with the overall shape of the data set.

(3) The 'transient' X-ray periodicities reported for 4U1700–37 could be due to occasional trains of quasi-periodic events in the flaring activity of the source. This variability may be interpreted as the result of shot noise; from the periodograms obtained by Fourier analysis of the Copernicus data we deduce a characteristic exponential decay time<sup>11</sup> of  $\sim 15$  min.

Much more observing time is required to satisfactorily clarify the temporal behaviour of 4U1700–37. In particular the data are too limited to precisely evaluate the third alternative proposed.

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## X-ray observations of AM Herculis from OSO 8

THE white dwarf binary system AM Herculis (2A1815+500) has been observed in X rays at both low energies ( $E \leq 10$  keV)<sup>1–3</sup> and higher energies<sup>2,4,5</sup>. The exact shape of the spectrum, particularly at the higher energies, has yet to be determined. We present here results from the high energy scintillation spectrometer on OSO 8. These are combined with results published elsewhere<sup>6</sup> obtained concurrently with the proportional counter on the same satellite, thereby giving for the first time coincident observations of AM Her over the range 2–250 keV.

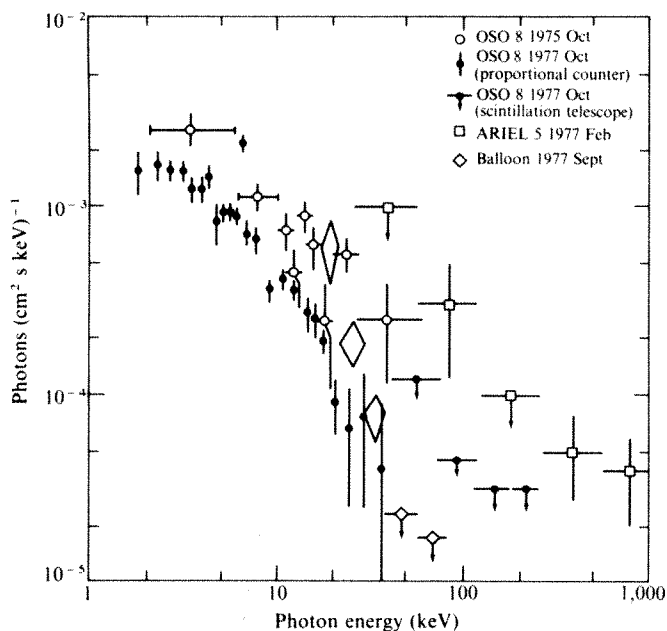
The OSO 8 instruments used to observe AM Her consist of a proportional counter<sup>7</sup> covering the photon energy range 2–40 keV, and a high energy scintillation spectrometer<sup>8</sup> covering the range 20–250 keV.

The source was observed during the period 1–10 October 1977. The results presented here from the scintillation spectrometer cover the whole period, whereas the proportional counter spectrum is based on 'quick-look' data taken during  $\sim 20\%$  of the period. The composite OSO 8 spectrum is shown in Fig. 1. Also shown for comparison are the spectrum of Staubert *et al.*<sup>5</sup> obtained on 20 September 1977, the Ariel 5 results<sup>4</sup> obtained during the period 18–22 February 1977 and the OSO 8 proportional counter spectrum obtained during the period 11–12 October 1975<sup>2</sup>. AM Her is an eclipsing binary system and all the results except those of Staubert *et al.*<sup>5</sup> have been averaged over the whole binary cycle. Since Staubert *et al.*<sup>5</sup> observed it for only part of a cycle, their results have been normalised by a factor of 0.83. This factor was obtained by comparing the phases of their observation periods with the 10–60 keV light curve of Swank *et al.*<sup>2</sup>.

Variations in the spectral shape and intensity are apparent in the results up to 100 keV. Even if the Ariel 5 results<sup>4</sup> in this energy range are not considered because of their low significance ( $\sim 1.7$  s.d.), there remains a statistically significant difference between the results of Staubert *et al.*<sup>5</sup> and OSO 8 taken only 10–20 d apart. If a thermal bremsstrahlung spectrum, including the modified Gaunt factor, is fitted to each data set, then apparent order of magnitude changes in temperature seem to be occurring (see, for example, the temperature of  $\leq 18$  keV found by Staubert *et al.*<sup>5</sup> to their 1977 observations and the temperature of 170 keV fitted by Swank *et al.*<sup>2</sup> to their 1975 OSO 8 data).

The X-ray emission of AM Her has already been shown<sup>9</sup> to vary in soft X rays ( $E \leq 10$  keV) by a factor of approximately four from one binary cycle to the next. The results summarised here indicate that changes in spectral shape also seem to be occurring. Although the changes in intensity are certainly real, the variations in temperature are not so well defined. The results from OSO 8 illustrate the confusion. In 1975 a single temperature fit to the data was found to be statistically unacceptable<sup>2</sup>, whereas in 1977 the opposite was true (Swank, personal communication). Furthermore, if the spectrum of Staubert *et al.*<sup>5</sup> is extrapolated to lower energies, then the flux in the 2–5 keV range would be at least one order of magnitude greater than that ever observed. Consequently, the latter results and the 1975 OSO 8 results suggest a spectral break being present, possibly at about 15 keV, on these occasions. The 1977 OSO 8 results, however, require no such feature.

If the spectrum of AM Her is definitely shown to possess a spectral break on some occasions, then at these times it will be similar to that seen from Her X-1 (ref. 10) in which a break is observed around 25 keV. The reason for the break in the Her X-1 spectrum is thought to be the modification of the Thomson cross-section in the intense ( $10^{13}$  G) magnetic field of the neutron star. The magnetic field of the white dwarf in AM Her is, however, believed to be of the order of  $10^8$  G from optical



**Fig. 1** Measurements of the X-ray spectrum of AM Her. The OSO 8 data below 50 keV come from the GSFC proportional counter experiment<sup>2,6</sup>, while the data above 50 keV come from the present work. The Ariel 5 data is from ref. 4, and the balloon data from Staubert *et al.*<sup>5</sup>. Upper limits are indicated at the two standard deviation level.

polarisation measurements<sup>12</sup>. The calculations of Canuto *et al.*<sup>11</sup> indicate that this would not be high enough to affect the X-ray photons. Consequently, if a break in the AM Her spectrum is subsequently confirmed, then some modified or alternative theory is required to explain it.

Finally, the upper limits obtained above 50 keV by Staubert *et al.*<sup>5</sup> and the present work make it unlikely that a  $\gamma$ -ray tail, such as that seen at the 3.2 standard deviation level in the Ariel 5 data<sup>4</sup>, existed on these occasions. In view of the possible spectral changes suggested by the results summarised in this work, however, the existence of such a feature on other occasions cannot be excluded.

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## Near IR surface brightness of southern galactic plane

NEAR IR surface of the Galaxy has been observed in balloon experiments<sup>1-6</sup>. The stellar distribution in the inner Galaxy thus obtained is related to the distributions of H I clouds, CO clouds,  $\gamma$  rays, OH-IR sources and so on<sup>7</sup>. These observations were restricted to the northern sky, and we have recently attempted to use balloon observations from Australia to study the overall structure of the Galaxy more completely. We found that (1) humps for 2.4- $\mu$ m surface brightness are asymmetric with respect to the galactic centre and attributed to the spiral structure; (2) the 2.4- $\mu$ m galactic plane is distorted from the flat plane in the regions around the most pronounced humps; (3) IR sources are highly concentrated in the plane to compensate interstellar absorption; and (4) the central region shows a complex structure.

The instrument we used is similar to that described by Ito *et al.*<sup>2</sup>. Incident radiation was focused by a 10-cm,  $F1.0$  silicon lens onto 11 PbS detectors, with fields of view of 0.5°, 0.8° and 1.7° at 2.4  $\mu$ m, and 2.0° at 3.4  $\mu$ m. The whole telescope system was cooled by liquid nitrogen to reduce the thermal background noise. The optical axis was fixed at an elevation angle of 40° and regularly scanned in azimuth through a 60° range centred on the galactic plane.

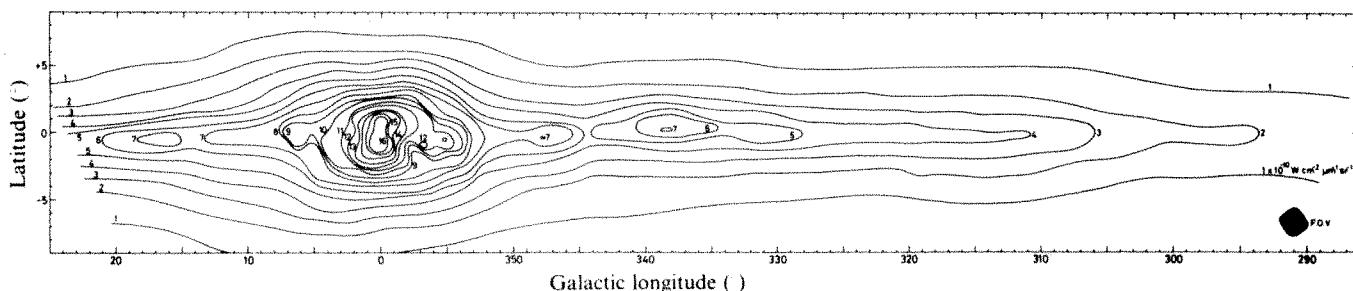
The payload was launched on 25 April 1978 at the Australian Balloon Launching Station, Mildura, Victoria, Australia. The instrument functioned satisfactorily during the whole flight, and the telescope surveyed the galactic plane between  $l = 290^\circ$  and  $20^\circ$ . During the azimuthal scans we observed several bright stars which enabled us to obtain the absolute flux value at 2.4  $\mu$ m within  $\pm 10\%$ , and to determine the latitude of the telescope within  $\pm 0.2^\circ$ . The absolute flux value at 3.4  $\mu$ m was obtained with lower accuracy.

The contour map obtained with the 1.7° field of view at 2.4  $\mu$ m is shown in Fig. 1, in which the contributions of bright stars,  $\alpha$ -Cen,  $\gamma$ -Cru, and  $\epsilon$ -Mus, are subtracted. The extended disk structure with about 5° width and some humps are observed. In addition, complex features are well resolved in the galactic centre region because of a low airglow level attained at a high elevation angle. Maps obtained with finer fields of view show similar features but a more pronounced structure near the galactic centre. This implies that the derived surface brightness results from an extended source and is not influenced by nearby bright stars; this can be partly confirmed using the data of the 2.2  $\mu$ m survey of stars<sup>8,9</sup>.

Figure 2 shows the longitudinal distribution of the peak surface brightness near the galactic equator at 2.4  $\mu$ m observed with the 1.7° field of view. The error due to detector noise and a variation of the airglow level is as small as  $3 \times 10^{-11} \text{ W cm}^{-2} \mu\text{m}^{-1} \text{ sr}^{-1}$ .

Humps at  $l = -13^\circ, -22^\circ, -45^\circ$  were first found in this experiment; similar humps at  $l = 16^\circ, 27^\circ, 47^\circ$  had been detected during previous observations. The hump at  $l = 27^\circ$  indicates a strong concentration of IR sources in the region at  $\sim 5$  kpc from the galactic centre; molecular clouds, H II regions and other young objects are also concentrated in this '5 kpc ring'. However, our present result indicates that the surface brightness is distributed not symmetrically, and suggests the spiral structure of the Galaxy. Although the velocity distribution of CO clouds does not clearly show the ridge of the spiral structure<sup>10</sup>, the 5-kpc ring may represent part of the bright galactic arm.

In Fig. 2, G and S indicate the tangential directions to the galactic arms modelled from the distribution of large H II regions<sup>11</sup> and from the density wave theory mainly based on H I observations<sup>12</sup> respectively. The agreement is not good over the entire galactic plane, the discrepancy being larger for the southern galactic plane. However, a pair of innermost humps at



**Fig. 1** The contour map of 2.4  $\mu\text{m}$  surface brightness with 1.7° field of view. The shaded area indicates the field of view used.

$l = 16^\circ$  and  $-13^\circ$  would indicate the existence of the dispersion ring. Observed asymmetry is essentially consistent with the ellipticity and the direction of the major axis in the model<sup>12</sup>.

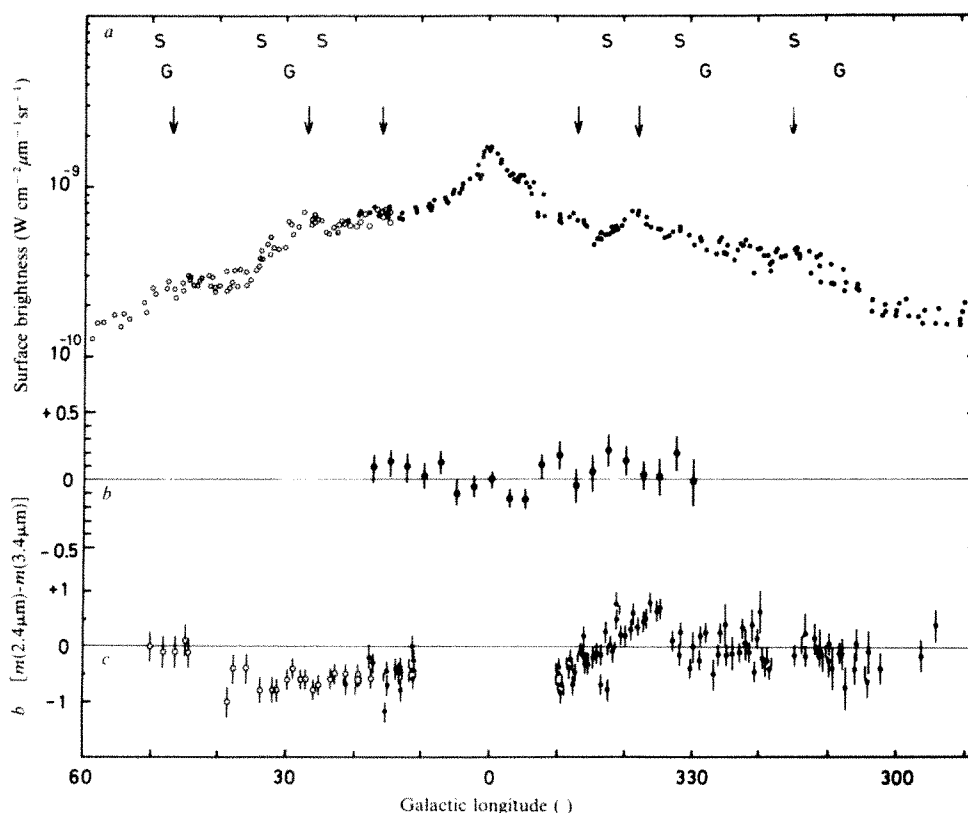
Figure 2b shows the relative colour,  $[m(2.4 \mu\text{m}) - m(3.4 \mu\text{m})]$  at the surface brightness peak plotted against longitude, with the normalisation to  $[m(2.4 \mu\text{m}) - m(3.4 \mu\text{m})] = 0$  at  $l = 0^\circ$ . A colour difference of  $\sim 0.3$  mag is found between the disk and galactic centre directions; that is, the disk is redder than the central region. The colour difference of 0.3 mag corresponds to the excess interstellar extinction of  $A_V \sim 10$  mag towards the disk direction over the galactic centre direction, consistent with the model of the distribution of interstellar dust<sup>7</sup>.

The latitude of the peak surface brightness for each scan is also plotted in Fig. 2c. Peak surface brightness is situated  $\sim 0.5^\circ$  below the galactic equator for  $10^\circ < l < 30^\circ$  and  $\sim 0.5^\circ$  above the galactic equator for  $335^\circ < l < 340^\circ$ . Note that these displacements seem to be associated with bright humps at  $l = 27^\circ$  and  $-22^\circ$ . For the northern galactic plane, similar displacements are

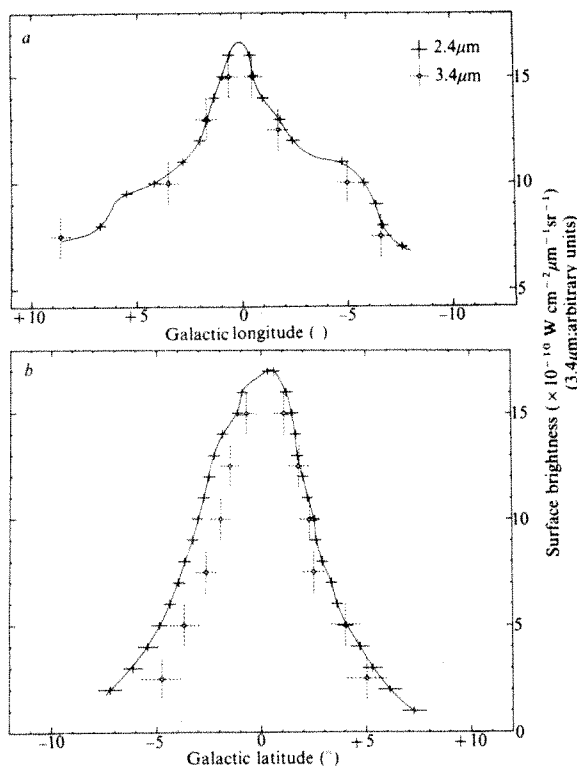
observed for the 5 kpc ring for CO clouds<sup>13</sup>, H II regions<sup>14</sup>, and OH-IR sources<sup>15</sup>.

Another interesting feature is the latitude distribution of surface brightness. Even for the finest field of view at 2.4  $\mu\text{m}$ , we do not find the absorbing feature which might be expected from a strong concentration of absorbing matter in the plane. This implies that the IR sources are also concentrated in the galactic plane, supporting the view that they are young objects, probably M supergiants. At 3.4  $\mu\text{m}$  the galactic plane is narrower than that at 2.4  $\mu\text{m}$ . This difference can be explained by weaker interstellar extinction at 3.4  $\mu\text{m}$ .

The galactic centre region reveals several interesting features. First, the 2.4  $\mu\text{m}$  plot within  $4^\circ$  from the galactic centre is asymmetric with respect to the galactic plane, similar to the tilted disk modelled from the H I observations<sup>16</sup>. Second, two features extend in longitude up to  $l = 7^\circ$  and  $-6^\circ$ . The feature at  $l \sim 355^\circ$  was detected by previous observations<sup>3,4</sup> as an extended IR source; its counterpart with a similar, though weaker, feature



**Fig. 2** The longitude distribution of the peak surface brightness at 2.4  $\mu\text{m}$  for each scan with 1.7° field of view. ●, The present result; ○, our previous observations<sup>1,2,4</sup>, where they have not been superseded by the present work. Arrows show the positions of characteristic humps. S and G indicate the tangential directions according to different galactic arm models. b, The longitude dependence of relative colour,  $[m(2.4 \mu\text{m}) - m(3.4 \mu\text{m})]$ , at the peak, normalised at  $l = 0^\circ$ . c, The latitude of the peak surface brightness for each scan.



**Fig. 3** *a*, The longitude distributions ( $b = 0$ ); *b*, the latitude distributions ( $l = 0$ ) of surface brightness at 2.4  $\mu\text{m}$  and 3.4  $\mu\text{m}$  on the galactic plane with 1.7° and 2° fields of view, respectively.

is first found at  $l = +6^\circ$ . This asymmetrical structure suggests that there exists a disk-like component on which the central bulge is superimposed. Third, there are two dark lanes intruding into the plane from below. The southern one is more pronounced and was detected by our previous observation<sup>4</sup>. These dark lanes may be partly explained by the dark clouds between the galactic centre and the Sun  $0.5^\circ$  below the  $b = 0^\circ$  plane<sup>13</sup>. Finally, Fig. 3 compares the 2.4 and 3.4  $\mu\text{m}$  data, and they show no appreciable colour variation with longitude, but an excess of 2.4  $\mu\text{m}$  flux for  $b < 0$ .

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## Stable 'pancake' distributions of low energy electrons in the plasma trough

WAVE-PARTICLE interactions have been recognised as the likely key to processes which control the dynamics of the Earth's magnetosphere and trigger auroral precipitation<sup>1</sup>. The GEOS 1 and GEOS 2 satellites carry wave and particle measuring instruments which permit a full exploration of interactions occurring in the equatorial magnetosphere out to a distance of 7  $R_E$  (ref. 2). The large variety of plasma wave phenomena has been reviewed by Southwood<sup>3</sup> and Christiansen *et al.*<sup>4</sup> have reported on emissions observed by GEOS 1. The geostationary position was expected to be an oasis of important wave-particle phenomena because it is in that region of the magnetosphere where cold plasma, originating from the ionosphere, mixes with hot plasma-sheet particles<sup>5</sup>. The large amount of data now emanating from GEOS 2 more than meets expectations, a number of relatively exotic particle distributions are being observed together with clearly associated wave spectra. Here, we report on one significant example.

The UCSD spectrometers aboard the ATS 5 and ATS 6 satellites yielded a magnificent data set for the particle environment at 6.6  $R_E$  (ref. 6). This, together with data from satellites in highly eccentric orbits (such as OGO 5 and Explorer 45), has led to an established picture of particle convection, substorm injection and subsequent dispersion, excursion of the plasmopause boundary and ring current evolution.

Theories of wave generation depend on the creation of instabilities and call for anisotropic particle populations; a distribution function  $f(E)$  with  $df/dv_{\perp} > 0$  is particularly sought. The inevitable loss cone is an obvious candidate source, it can be enlarged by pitch angle diffusion<sup>7</sup>; also the inward convection from the tail generates thermal anisotropy ( $T_{\perp} > T_{\parallel}$ ) but the key parameter is probably the ratio between the densities of 'cold' and 'warm' plasma components. Sufficient cold electrons are required for wave development but too many will damp the unstable mode<sup>8</sup>.

The suprathermal plasma analysers (SPAs) on GEOS 2 have regularly detected a population of electrons over 50 eV which are confined to pitch angles near  $90^\circ$ —a 'pancake' distribution. These particles can be observed near the geomagnetic equator for several hours a day but the dependence on local time is variable, as are the intensity profile and energy spectrum. GEOS also gives the first reliable measurements of cold plasma density near 6.6  $R_E$  and a preliminary description of these new results is presented here. Electrostatic emissions near the half harmonics of the electron-cyclotron frequency are commonly observed near the geomagnetic equator<sup>9</sup>, Gough *et al.*<sup>10</sup> present further evidence for observed correlations but a firm conclusion on cause and effect is not yet clear.

Figure 1 shows the GEOS spacecraft illustrating the position and viewing directions of the SPA sensors which incorporate simple hemispherical electrostatic analysers<sup>11</sup>. The isolated boom mounting of the sensor unit with the utilisation of a bias voltage to overcome the satellite floating potential (a few volts positive) allows the collection of cold protons, the complex sheath structure obviously influences the detected fluxes but in-flight calibration is available in the plasma frequency determination from the wave experiment<sup>4</sup>. The conducting surface of GEOS seems to eliminate the problem of differential charging



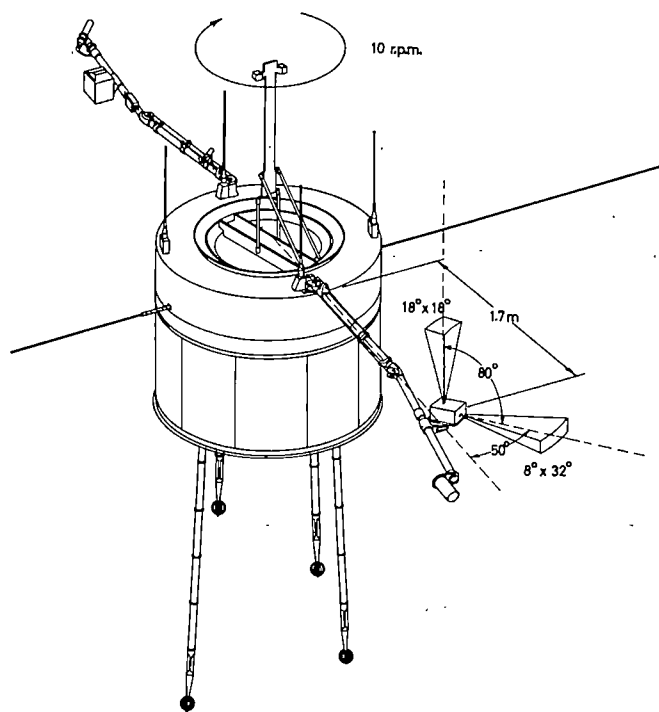


Fig. 1 GEOS satellite showing the position of the suprathermal plasma analysers.

seen on ATS<sup>12</sup> and this is crucial for these very low energy measurements. One analyser views parallel to the satellite spin axis but this is seldom magnetic field-aligned. In fact the axis of GEOS 2 is usually between  $10^\circ$  and  $15^\circ$  ( $\theta_B$ ) from the field direction and consequently the radial analyser, scanning pitch angles between  $(80 - \theta_B)$  and  $(80 + \theta_B)$  during a 6 s spin period, is ideally pointed to detect pancake distributions. Sixty-four energy channels ( $\Delta E/E = 0.11$ ), logarithmically spaced, cover the full range of 0.5–500 eV but below 20 eV satellite emitted photoelectrons mask the ambient fluxes. It takes about 3 min to obtain a full coverage of energy (20–500 eV) over the available pitch angles.

Figures 2 and 3 show the energy dependence of the pancake distribution by comparing three selected pitch angles ( $\alpha \leq 90^\circ$ )

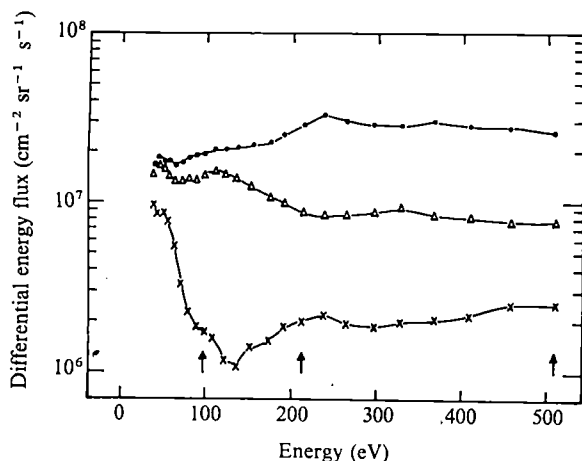


Fig. 2 Energy spectra for pitch angles:  $90^\circ$  (●);  $69^\circ$  (Δ);  $12^\circ$  (×) taken from detector viewing parallel to spin axis. (2 August 1978 at 5.05 UT;  $0.75^\circ$  N,  $8.8^\circ$  E)

and three selected energy channels. Count rates have been converted to differential energy flux and data from the parallel analyser ( $\alpha = \theta_B = 12^\circ$ ) are included to allow interpolation to small pitch angles. Although the form of the distribution is quite variable, these curves are typical; both the peak flux and the maximum anisotropy often appear below 500 eV, the anisotropy tends to vanish at energies less than 100 eV but here the photoelectrons become significant. The degree of anisotropy can be assessed by fitting a  $\sin^n \alpha$  function; where  $n$  takes a value of 10–20 at the peak the electrons are confined to pitch angles very close to  $90^\circ$ . The analyser acceptance angles (see Fig. 1) do produce some instrumental broadening of the feature. The

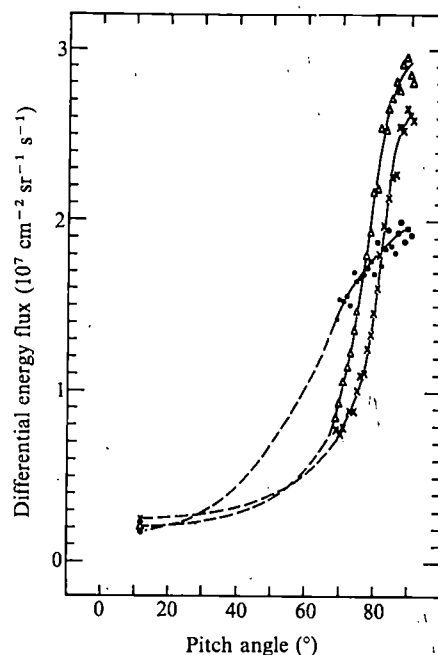


Fig. 3 Pitch angle distributions for energy channels: 211 (Δ); 509 (×); 98 (●) eV.

phase space density of the  $90^\circ$  electrons does not exhibit a positive slope when plotted against energy.

The pancake population is generally observed by GEOS 2 during the morning hours. Near local midnight the SPAs detect plasma-sheet electrons which are isotropic, the differential fluxes increase with energy but the maximum is obviously well above our 500 eV limit. Near noon or later, GEOS 2 regularly encounters a cold plasma enhancement with density  $N_i$  of  $50 \text{ cm}^{-3}$  or more. It is not always clear whether these are excursions into the plasmasphere or through detached regions. Between the two, in the morningside plasma trough, the  $\alpha = 90^\circ$  electrons can persist for many hours. To search for possible correlation with electrostatic wave emission, a period of 7 days in August 1978 has been studied and Fig. 4 summarises the results. The geomagnetic planetary index  $K_p$  reached 5 on 12 August but the other days were fairly quiet. The magnetic field briefly departed from a dipolar configuration on only three occasions;  $V$ ,  $D$ ,  $H$  components supplied by Mariani have been used to compute  $\lambda = \tan^{-1} [-V/2H]$  as an estimate of an effective magnetic latitude; this suggests that GEOS 2 was within  $3^\circ$  of the equator for most of the period. The cold plasma density was determined by using a sensor bias of  $-28 \text{ V}$  and calibrating maximum count rates with reference to appropriate plasma frequency measurements; no attempt has yet been made to analyse the spectra but it is clear that the temperature cannot be significantly greater than 1 eV. The plasmaspheric passages tend to be earlier than would be consistent with an evening bulge

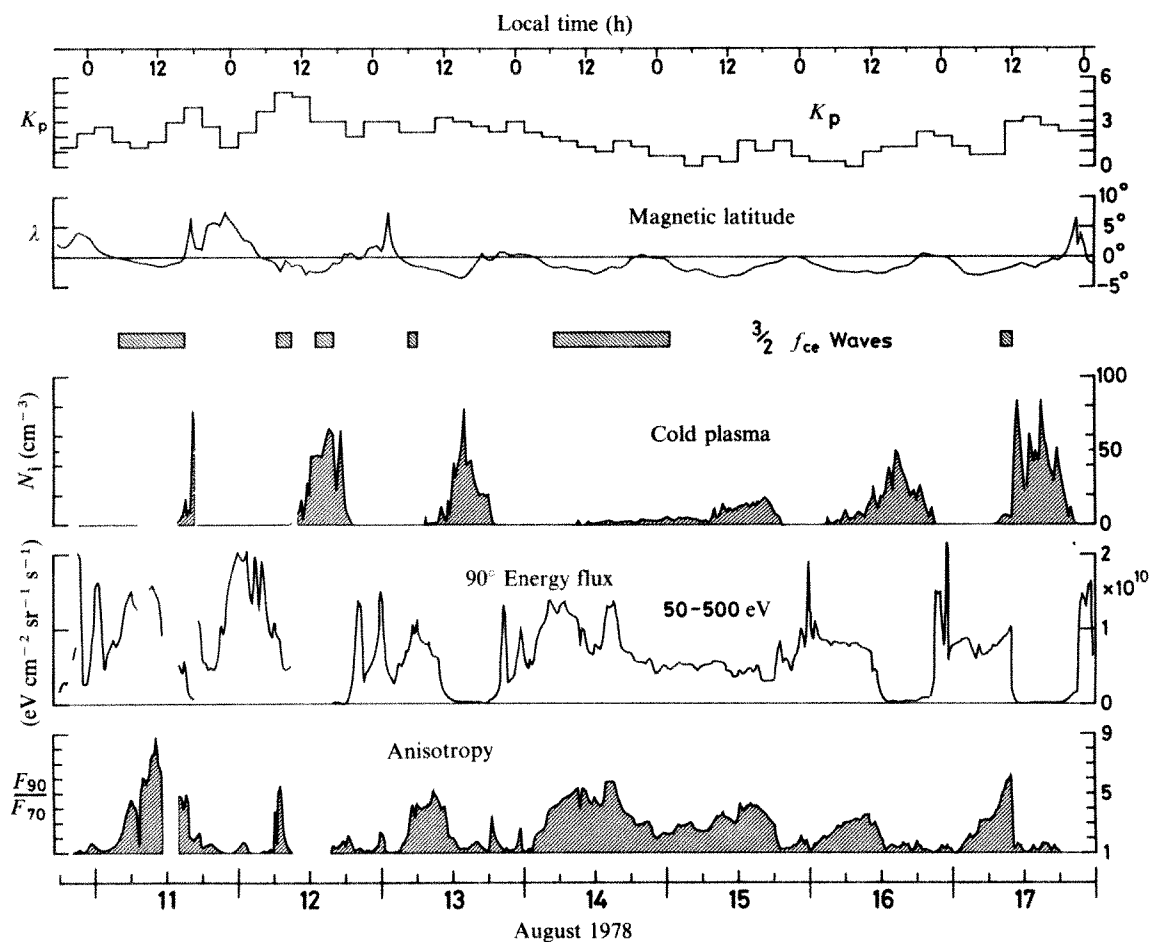


Fig. 4 One week's data from GEOS 2 showing the appearance of pancake electron distributions in relation to cold plasma enhancements and ' $3/2 f_{ce}$ ' emissions.

and  $N_1$  can be  $\sim 2 \text{ cm}^{-3}$  or more all around the orbit when  $K_p$  remains small. At such times the total energy flux for electrons between 50 and 500 eV shows that plasma-sheet interceptions can be very short or even non-existent. The flux for  $\alpha = 90^\circ$  divided by that for  $\alpha = 70^\circ$  provides an anisotropy index which shows the presence of the pancake distribution. The latter normally builds up during the morning hours and disappears at a plasmopause entry, there can be a sharp cut off as on 17 August but on the quiet days the pancakes persisted as  $N_1$  did not exceed  $10 \text{ cm}^{-3}$ . The growth of the anisotropic distributions is usually from 500 eV downwards in energy and it will be interesting to examine other GEOS data (S310) to find the high energy limit.

Detection of strong electron cyclotron harmonic emissions (' $3/2 f_{ce}$ ') is indicated on Fig. 4 by the identification of times for which the integrated spectral density between  $f_{ce}$  and  $2f_{ce}$  exceeds  $10^{-9} \text{ (V}^2 \text{ m}^{-2})$ . A high anisotropy index ( $>3$ ) when combined with an absence of dense cold plasma ( $<10 \text{ cm}^{-3}$ ) generally corresponds with strong wave generation. This striking coincidence of the pancakes and the ECH waves is a feature of all GEOS 1 and GEOS 2 data although there are a few obvious exceptions which need explanation. Gough *et al.*<sup>10</sup> describe the complexity of the wave spectra and introduce a classification as a starting point in ordering the data; evidence of interaction is clearly identified.

We have given an early announcement of a magnetospheric particle population which must be an important quiet-time feature of the equatorial plasma trough. It has not been reported previously; GEOS 2 is the first high altitude satellite, with suitable low energy particle detectors, stationed close to the geomagnetic equator. The pancake distributions almost certainly result from wave interaction; the observed electro-

static waves seem to be responsible but the interaction is not 'local' in that the drift path of the electrons is not along the satellite orbit. The stability of the consequent distribution and the inherent equatorial confinement could then determine the morphology of the class III waves<sup>10</sup>. A detailed study is under way and we will be disappointed if this is not matched by renewed activity on the part of theoreticians.

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## Interaction of electrostatic waves with warm electrons at the geomagnetic equator

WE present here observations of electron-cyclotron harmonic (ECH) waves occurring in the vicinity of the geomagnetic equator at 6.6 Earth's radii, and discuss their relevance to the morphology of electron distributions (with energies below 500 eV) observed simultaneously<sup>1</sup>.

The observations were carried out during the slow crossing of the geomagnetic equator (4–13 August 1978) by the GEOS 2 satellite, launched into a geostationary orbit on 28 July 1978. The earlier GEOS 1 satellite, which covered a wider latitude range in a non-geostationary orbit, showed that a particular class of dayside ECH wave emissions are confined within a couple of degrees about the geomagnetic equator<sup>2</sup>.

The ECH instability, characterised by emission in frequency bands between the harmonics of the electron gyrofrequency  $f_{ce}$ , has been observed in a variety of spectral forms by a number of magnetospheric satellites<sup>3–5</sup>. However, because the GEOS series combines unique active plasma diagnostic techniques<sup>6–8</sup> with natural wave detection<sup>9</sup> the observed natural emissions can be much more readily related to local plasma parameters than can results from earlier spacecraft.

It has been shown theoretically that the ECH instability can be explained in terms of a 'basic' two-component plasma model<sup>10–15</sup>: the free energy for wave amplification is provided by a hot loss-cone component ( $T_h \sim 100$  eV) while a second cool component ( $T_c \sim 1$  eV) presumably of ionospheric origin, aids destabilisation. For a wide range of values of the ratio of the cool to the hot electron density,  $N_c/N_h$ , the dominant instability frequency occurs close to the cool plasma upper hybrid frequency  $f_{uhc} = (f_{pc}^2 + f_{ce}^2)^{1/2}$ , where  $f_{pc}$  is the cool plasma frequency. Other spectral features can occupy gyroharmonic bands below and above  $f_{uhc}$ .

Because the value of the cold plasma density strongly

influences the observed spectral characteristics of the ECH waves, it has been used as a guideline for classification schemes<sup>14</sup> including the one given below.

Note that at the geostationary orbit  $f_{ce}$  lies typically between 2 and 3 kHz, while  $f_{pc}$  spans a wide range from below  $f_{ce}$  up to above the maximum observable frequency of 77 kHz.

A spectrogram of wave data from GEOS 2 for the period covering the last hours of the 6 August and the full orbit for the 7 August 1978 (Fig. 1) shows the four main classes of ECH emissions detected.

Class I emissions, characterised by a single frequency band just above  $f_{ce}$  (typically  $1.2 f_{ce}$ ), occur around local midnight where the spacecraft encounters the plasma sheet, dominated by hot plasma convected in from the geomagnetic tail ( $f_{pc} < f_{ce}$ ) and can be seen about 00.00 UT and 22.00–24.00 UT on the 7 August.

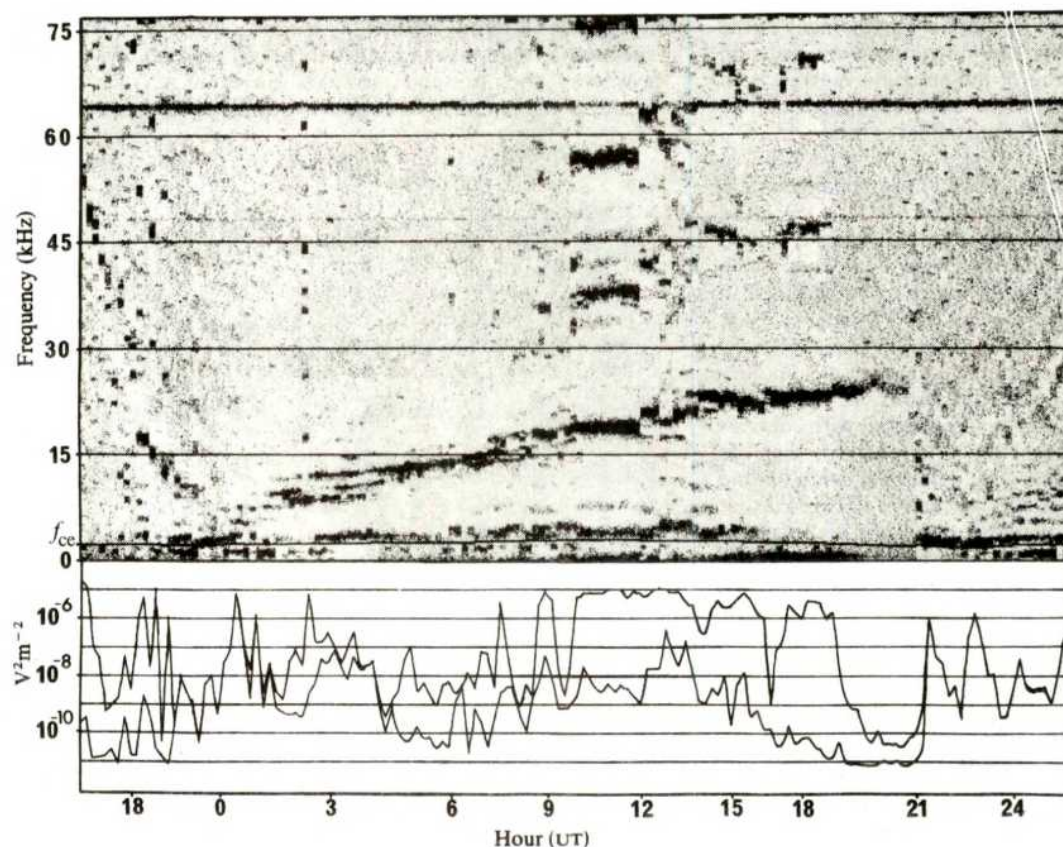
Class II emissions are briefly seen as the satellite moves into the morning sector, and the cold plasma density increases. Here the dominant intensity of the emission still occupies the lowest gyroharmonic band ( $f_{pc} \sim f_{ce}$ ) but is accompanied by weaker, higher harmonic structures.

Class III emissions, can be seen for a long period after 01.00 UT, when the cold plasma density rises ( $f_{pc} > 2f_{ce}$ ). The dominant frequency is sometimes banded in a complex fashion, and increases to  $\sim 25$  kHz around 19.00 UT, with accompanying  $(n + \frac{1}{2})f_{ce}$  emissions between low gyroharmonics. On board plasma diagnostics show that during this period  $f_{uhc}$  is within 1 kHz of the dominant emission band.

Class IV emission<sup>10</sup>, is a weak emission in the vicinity of the  $f_{uhc}$  which is observed after 19.00 UT, when the low harmonic signals at  $\sim 3/2, 5/2 f_{ce}$  disappear.

This particular orbit does not show the usual encounter with the denser evening bulge, but the typical class IV and class III emissions associated with it can just be seen during the last hours of the 6 August (extreme left of Fig. 1).

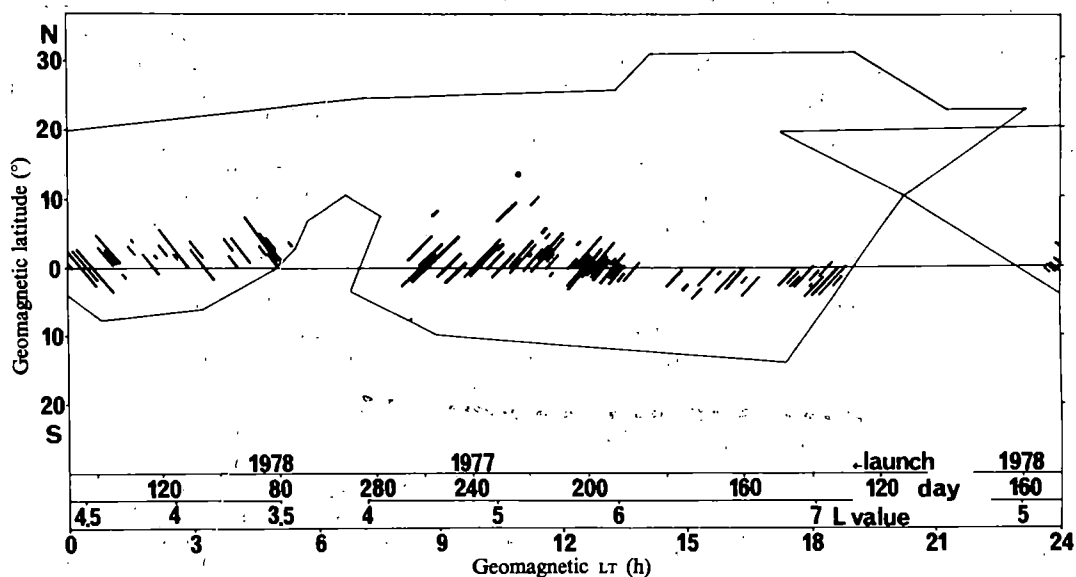
Some feeling for relative emission intensities can be gained from the plot at the bottom of Fig. 1. The upper trace is the integrated spectral density for frequencies above  $f_{ce}$ , and the lower trace is the spectral density integrated over the range from



**Fig. 1** Grey-scaled GEOS 2 electric field data for 7 August 1978 plotted with frequency against Universal time. The lower trace shows the integrated spectral densities for all electrostatic waves above the gyrofrequency. The lowest line is that from  $f_{ce}$  to  $2f_{ce}$  only. Geomagnetic LT is UT + 1 h.



**Fig. 2** All GEOS 1 observations (excluding storm periods) of type III electrostatic emissions plotted as a function of geomagnetic latitude and geomagnetic local time. Closed line is limit of observations. McIlwain  $L$  value and day number at which the satellite crossed geomagnetic equator are indicated below.



$f_{ce}$  to  $2f_{ce}$  only. Note that the spectral density peaks twice per orbit, once around midnight for class I ( $E \sim 1 \text{ mV m}^{-1}$ ) and once in the morning-midday region for class III, when the signals are so strong ( $E > 3 \text{ mV m}^{-1}$ ) that the wave detector saturates and harmonic generation can be observed.

The distribution of class III events (enhanced upper hybrid with  $3/2 f_{ce}$  features) reported recently for dayside emissions<sup>2</sup> is shown in Figs 2 and 3, using data from GEOS 1 (perigee 2,000 km, apogee 42,000 km,  $-10^\circ < \lambda_m < 30^\circ$ , taking in excess of a year to survey all local times). This analysis covers 14 months of satellite operation, and class III events were recorded during the vast majority of equator crossings (Fig. 2) in which the wave analysers were in the appropriate observing modes. Those emissions which occurred far from the model field equator were accompanied by the largest differences between the field observed at the spacecraft and the modelled geomagnetic field, suggesting that in these cases the field lines were distorted.

Figure 3 further describes the equatorial confinement of the class III emissions, by showing the distribution of emission widths (Fig. 3a) and the location of the emission centres, both plotted as a function of geomagnetic latitude. Approximately 75% of the emissions were  $< 4^\circ$  in latitudinal extent, and 75% were centred between  $-2^\circ < \lambda_m < +3^\circ$ .

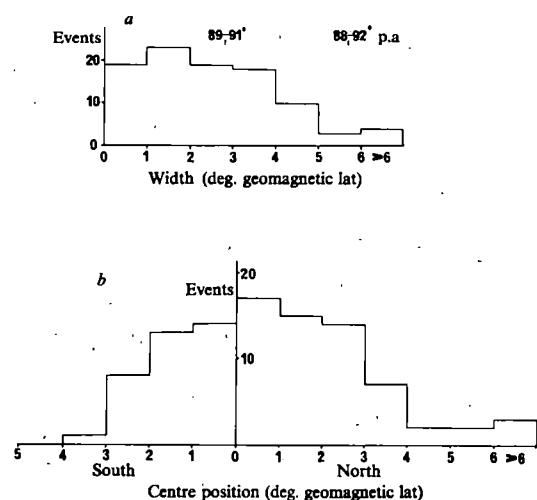
In August 1978 GEOS 2 drifted slowly through the geomagnetic equator towards its present position of  $\lambda_m = -3^\circ$ , and during this period Wrenn, Johnson and Sojka<sup>1</sup> report frequent observations of remarkable electron distribution features, which show electrons in the hundreds of eV range to be confined to pitch angles within a few degrees of  $90^\circ$  ('pancake' distributions). Figure 4 summarises 10 d of GEOS 2 observations around the geomagnetic equator, and compares plots of hourly means of the spectral density of ECH waves, versus hourly means of the anisotropy index<sup>1</sup> for warm electrons with energies  $< 500 \text{ eV}$ . These points incorporate all data received when the two experiments (wave and particle detectors) were in the appropriate operating modes. A striking feature of Fig. 4 is that as the ECH spectral density increases there is a well defined maximum of observable anisotropy index. In particular this behaviour is strongly related to that of class III emissions (black dots) which, as pointed out above, occur mainly between 04.00 and 13.00 UT. The narrow pitch angle distributions therefore tend not to occur in the midnight sector where class I and II emissions predominate. Furthermore, from a survey of GEOS 1 data they show the same sort of equatorial confinement as class III emissions<sup>1</sup>.

In seeking an explanation for these observations it is important to note that the combination of nightside convection of plasma sheet electrons with collisional diffusion during subsequent convection through the dayside, cannot produce

such highly pitch-angle peaked distributions. Inward convection can produce a loss-cone distribution, and therefore a source of free energy for ECH wave amplification<sup>16</sup>.

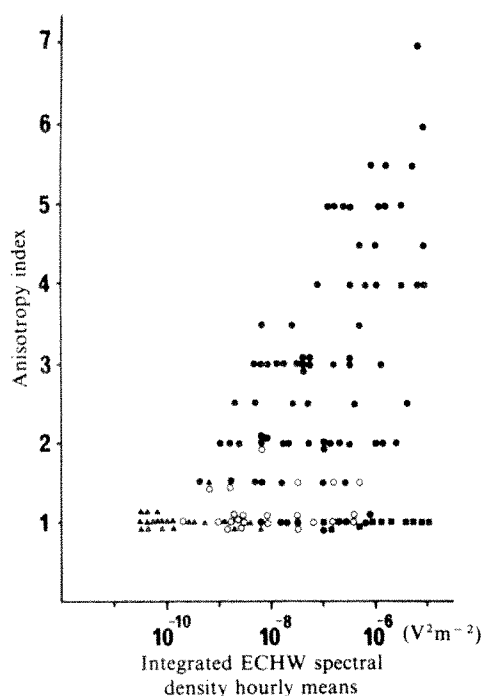
In the amplification process, resonant electrons, which satisfy the condition  $\omega - k_{\parallel} v_{\parallel} = \pm n \omega_{ce}$  are scattered in velocity space, the important exceptions being those with small  $v_{\parallel}$  (that is large pitch angles), which are relatively unaffected. Quasilinear theories show this clearly<sup>17</sup> and predict among other things, that for wave fields of  $\sim 1 \text{ mV m}^{-1}$  hot electrons can be rapidly scattered into the loss cone<sup>13</sup>.

We suggest that the 'pancake' distributions observed in conjunction with strong ECH wave activity are a remnant feature of the electron distribution, resulting from this interaction. Their evolution can be pictured as beginning with the class I emissions which are amplified by electrons freshly convected into the nightside magnetosphere, and progressing through further wave-particle interactions during the subsequent convection of the electrons around into the dayside magnetosphere. Since the pancake distributions can contribute to further destabilisation of the waves<sup>18</sup>, their presence may also be a significant factor in the equatorial confinement of class III emissions.



**Fig. 3** a, Distribution of widths of GEOS 1 observed type III emission in degrees geomagnetic latitude. Also indicated is the equivalent equatorial pitch angle range assuming the centre of the emission is at the minimum B equator. b, Distribution of centres of type III emissions in degrees geomagnetic latitudes from field model equator<sup>20</sup>.





**Fig. 4** GEOS 2 Hourly means of the integrated spectral density above the gyrofrequency plotted against the mean anisotropy of electrons below 500 eV between 14.44 UT on 4 August and 21.27 on 13 August. Class of emission: ○, class I and II; ●, class III; ▲, class IV; ■, class IV plasmasphere.

The role of ECH wave-particle interactions in providing the electron component of diffuse auroral precipitation has been much discussed in recent years<sup>3,17,19</sup>. We hope to extend our studies into this area shortly.

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## Contribution of volatile petroleum hydrocarbons to the organic carbon budget of an estuary

STUDIES on oil pollution of the marine environment have received considerable attention<sup>1</sup>, especially work involving the effects of oil spills resulting from tanker accidents such as the *Amoco Cadiz*<sup>2</sup>. Very little attention, however, has been devoted to assessing the importance of low level, continuous, inputs of petroleum hydrocarbons to productive estuarine environments. Investigations of hydrocarbon contamination of estuaries and rivers have concentrated mainly on the impact of non-volatile hydrocarbons<sup>3</sup> (that is, those hydrocarbons having more than 15 carbon atoms) rather than the volatile hydrocarbons emanating from sewage discharges and industrial effluents such as petroleum refineries. We discuss here the occurrence and possible fate of these volatile hydrocarbons in estuarine systems.

During research in determining an organic carbon budget for Southampton Water estuary, the seasonal fluctuations in planktonic respiration and photosynthesis were studied. Characteristically, we observed a pronounced seasonal cycle in planktonic photosynthesis with intense activity in the mid-summer months and little in the winter. In contrast, planktonic respiration showed a less pronounced seasonal change, respiration being sustained through the winter months (Fig. 1). It has been argued that the winter respiration is supported by the external inputs (sewage, rivers and industrial effluents)<sup>4</sup> and this would require a daily input with a biological oxygen demand (BOD) equivalent to 30-40 tonnes of O<sub>2</sub> per day. It may be seen that cumulative 5 d BOD at 15 °C is of this order (Table 1). The largest single industrial input to the estuary is the oil effluent from the Esso refinery at Fawley (the largest refinery in the UK with a refining capacity of 20 Mtonne yr<sup>-1</sup>). The refinery effluent accounts for 75% of the total industrially derived carbon discharged to the estuary yearly<sup>4</sup>.

A detailed study has been made of the non-volatile fraction of the refinery effluent and the role and fate of these compounds in the estuarine environment<sup>5</sup>. These studies showed that the non-volatile fraction, the most commonly studied part of oil, could not account for the observed respiration rates. This led us to hypothesise that the volatile hydrocarbon fraction, which usually receives little attention, may play an important role in, at least, this estuarine ecosystem.

Previous work on the biodegradation of petroleum hydrocarbons has been carried out using standard microbiological techniques<sup>6</sup> and it is widely accepted that marine microbial organisms can degrade oil<sup>7</sup>. The quantity and diversity of oil degraders present in marine systems are more likely to be higher for areas with continuous hydrocarbon discharges than pristine environments<sup>8</sup>. The likelihood of high numbers of petroleum utilising bacteria would be even greater for estuaries which contain high concentrations of other heterotrophs<sup>9</sup>. One would therefore expect that with such a large continuous input of petroleum hydrocarbons from the Fawley refinery over the past 25 yr, there would be a very significant resident population of specialised oil degraders at the refinery outfall area as well as in the estuary itself.

To investigate the degradation of the non-volatile fraction of the refinery effluent, we used large glass containers and monitored hydrocarbon concentrations using gas, solid-liquid<sup>10</sup> and thin layer<sup>11</sup> chromatography. Quantification of compounds that were separated into broad categories of compound type was accomplished by combustion in a total carbon analyser<sup>5</sup>.

The average total non-volatile hydrocarbon content of the refinery effluent determined by the above method was 3 mg C per l, giving a daily input of hydrocarbons of about 1 tonne C per day. The results of our biodegradation experiments indicated that only 34% of the non-volatile hydrocarbon fraction was degraded at 15 °C over the 5 d BOD period with more

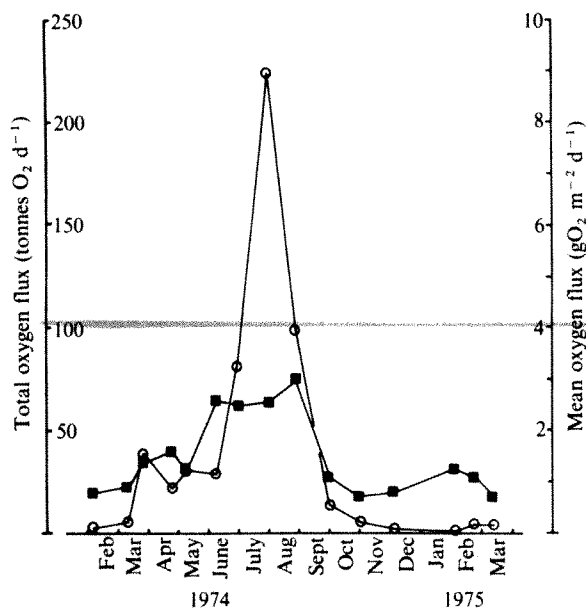


Fig. 1 Oxygen flux due to respiration and photosynthesis for Southampton Water estuary. ○, Gross photosynthesis; ■, respiration 1974–75.

than half the loss from the experimental containers attributable to evaporation processes. Assuming a respiratory quotient of 0.66 for the respiration of hydrocarbons, one may estimate the BOD stemming from the oxidation of the non-volatile fraction to be in the vicinity of 1 tonne  $O_2$  per day. Thus it seems that the degradation of the non-volatile fraction of the refinery effluent could not account for the high BOD of 40–100  $mg O_2$  per l of refinery effluent nor could it account for the high BOD in the estuary during the winter months. Due to the possible importance of this volatile fraction, we attempted to obtain more information on the compounds in this fraction of the refinery effluent.

We analysed the refinery effluent by the standard API method 733–758 for refinery waste water used by the refinery for its monitoring programme. This involved quantification of a carbon tetrachloride extract by IR spectrophotometry at  $2,930\text{ cm}^{-1}$  against a standard slop oil collected from the API gravity separators within the refinery complex. The results of these analyses indicated that the estuary received an average loading of 8–10 tonne of material per day as determined by this method. When the carbon tetrachloride extracts were evaporated at  $40^\circ\text{C}$  under vacuum and then redissolved in carbon tetrachloride and measured as before, the levels returned to 1–2 tonne per day. This was consistent with the results determined by the TLC/combustion method described previously. Gas chromatograms of the pre-evaporated and evaporated fraction are given (Fig. 2). Much of the extremely volatile material (hydrocarbons having 5 or less carbon atoms) is masked by the large solvent front at the start of the temperature programme. Ultraviolet (UV) analysis at 254 nm against the same standard

indicated that the removal of UV absorbing material (mainly aromatics and olefins) was similar in magnitude to those compounds absorbing at the  $2,930\text{ cm}^{-1}$  region of the IR. This tentatively indicated that the refinery effluent contained both aliphatic and aromatic compounds in the volatile fraction.

In an attempt to characterise the volatile fraction in more detail we used a cryogenic trapping system<sup>12</sup>. Volatile compounds were trapped in loops cooled by solid  $CO_2$  and analysed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). This yielded a wide range of volatile *n*-alkane and aromatic hydrocarbons. Aromatics such as benzene, toluene and xylene were present in concentrations of 120, 211 and  $562\text{ }\mu\text{g l}^{-1}$  respectively, indicating the importance of these compounds to the whole refinery input.

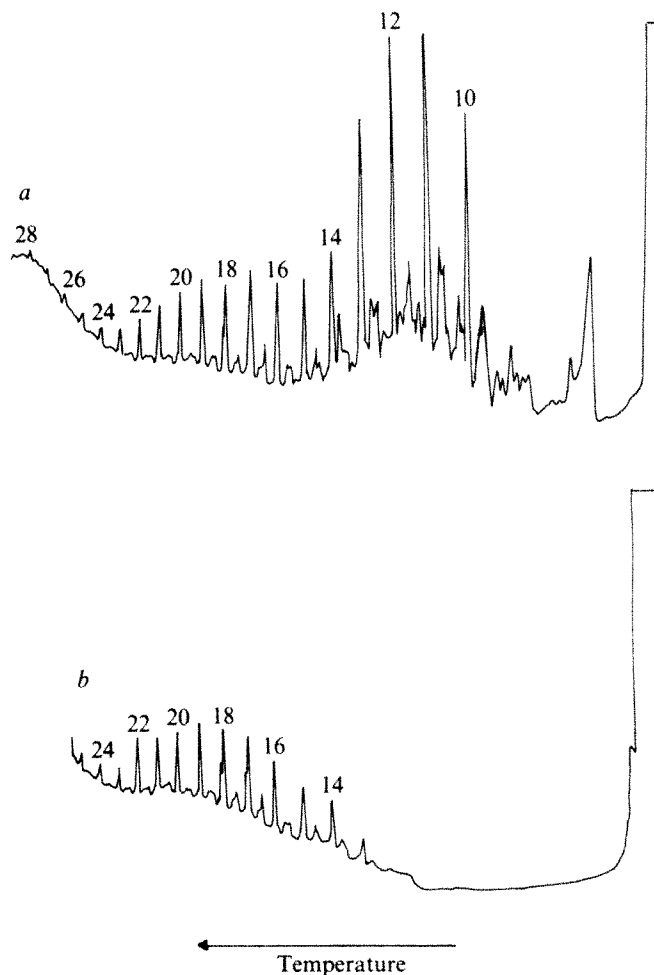


Fig. 2 Gas chromatograms of a, the total (volatile + non-volatile) compared with b, the non-volatile hydrocarbon composition of refinery effluent.

One would expect the residence time of these compounds in the estuarine environment to be similar to that of oceanic environments, aside from the obvious possibility of rapid biodegradation due to increased microbiological activity. For example, orders of magnitude changes in water concentrations of ethane, propane, butane and pentane were found over baseline in the proximity of an oil slick<sup>13</sup>. Sackett and Brooks also report volatile hydrocarbons present 8 km from an underwater drilling rig; concentrations of ethane alone were as high as  $0.3\text{ mg l}^{-1}$ .

The solution mechanism of these volatile hydrocarbons is dependent on the solubilities of the compounds in water relative to their vapour pressures. Also, as molecular weights increase more material is partitioned into the gas phase<sup>14</sup>. With petroleum hydrocarbons, partitioning into the gas phase decreases in the order of *n*-alkanes, olefins, cyclo-alkanes and

Table 1 Sources of organic material in Southampton Water estimated for 1974<sup>17</sup>

	Annual input (tonne C $\text{yr}^{-1}$ )	Percentage of total annual organic input	Daily winter BOD
Phytoplankton	3,000	23%	<5.0
Rivers	1,400	11%	4.2
Sewage	1,580	12%	3.2
Industry (Petroleum 75%)	6,800	53% (Petroleum 40%)	37.0

River input sampled upstream of major sewage and industrial outfalls.

aromatics<sup>15</sup>. McAuliffe<sup>15</sup> states that these volatile hydrocarbons remaining in the water column are eventually removed through biodegradation; we feel that this is also the fate of volatile hydrocarbons in Southampton Water.

In conclusion, the data that we have accumulated during 3 years on Southampton Water estuary suggest that the volatile compounds found in industrial effluents play a significant part in the whole carbon balance of the estuarine ecosystem. The importance of volatile fractions of petroleum hydrocarbons with regard to industrial discharges to estuaries has, to some extent, been overlooked in the past. There is evidence that these hydrocarbons, especially the aromatic component, are toxic to marine life<sup>16</sup>. These considerations indicate the need for more work in valuable estuarine areas to understand the occurrence, fate and impact of these volatile compounds.

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## Transformation of goethite to maghaemite in CsI disks

THE formation and stability of  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> (maghaemite) is still a puzzle, as this oxide is metastable with respect to its isomorphic form  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> (haematite). However, there are increasing reports about its natural occurrence in certain soils and sediments (see ref. 1 and refs therein). Maghaemite, like magnetite, Fe<sub>3</sub>O<sub>4</sub>, is ferrimagnetic and its artificial occurrence may therefore be important in carrying out magnetic concentrations in various processes of iron ores industry<sup>2</sup>; as well as finding widespread application in the electronic industry<sup>3</sup>. The artificial preparation of maghaemite is usually carried out by low temperature dehydration of lepidocrocite<sup>4</sup>,  $\gamma$ -FeOOH, or careful oxidation of powdered magnetite<sup>5,6</sup>.

Goethite,  $\alpha$ -FeOOH, heated in the presence of organic matter to 300°C, may also be transformed into maghaemite<sup>7,8</sup>. The optimal conditions for obtaining maghaemite in soils is by heating them in nitrogen, then in air at about 550 °C, which is the

**Table 1** Widths at half heights (in  $2\theta$  units) of characteristic X-ray diffraction peaks of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub>

<i>hkl</i>	<i>d</i> (Å)	Haematite	Protohaematite (a)*	Protohaematite (b)†
		NT	PT	ET
012	3.688	0.24	0.47	0.63
104	2.703	0.24	0.47	0.71
110	2.518	0.27	0.31	0.39
113	2.209	0.27	0.39	0.47

NT, not transformed to maghaemite; PT, partly transformed to maghaemite; ET, easily transformed to maghaemite.

\*Protohaematite (a) is obtained by heating goethite powder to 450 °C.

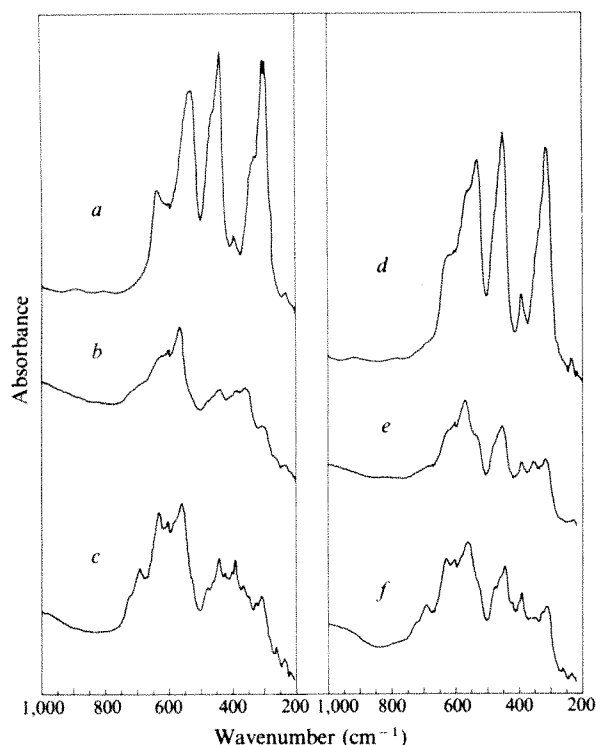
†Protohaematite (b) is obtained by heating goethite in CsI disk to 450 °C.

temperature at which the organic matter most readily produces a reducing environment<sup>9</sup>. When heating the soils in air the formation of maghaemite is negligible. The mechanism suggested for the last reactions in soil, is as follows<sup>1</sup>: gases such as carbon monoxide, produced by the combustion of soil organic matter, reduces finely divided iron oxides and hydroxides to magnetite and on cooling the latter may be subsequently oxidised to maghaemite when air enters the matrix. Normally, thermal treatment of goethite at temperatures 200-600 °C in the absence of organic matter results in a poorly crystalline form of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> which is defined here as protohaematite and which at higher temperatures is converted into the well ordered crystalline variety of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub>, known as haematite. We show here that maghaemite can be obtained by heating goethite or protohaematite in CsI disks in the absence of organic matter. IR spectroscopy has been previously used successfully to differentiate unambiguously between magnetite, maghaemite and haematite<sup>3</sup>, as well as between protohaematite and haematite<sup>10</sup>.

In the present study the transformation of synthetic goethite to maghaemite in CsI disks was followed by IR absorption spectroscopy and supported by magnetic evidence. Disks of 13 mm diameter were prepared by hand grinding 0.5-1.5 mg synthetic goethite in 400 mg CsI and pressing at 30 ton for 30 s. For the reaction to occur, every disk must be reground and re-pressed at least three times. The optimal conditions were: heating the disks to 250 °C for one night and then at 500 °C during several nights. Alternatively, the disks were directly heated to 500 °C, but in the latter case the results were less satisfactory. Each day the disks were re-pressed and IR spectra were recorded at ambient temperature using a Perkin-Elmer 283 IR spectrophotometer. Some representative IR spectra are shown in Fig. 1.

The spectrum obtained after heating the goethite disk for one night at 250°C is characteristic of protohaematite (Fig. 1a). This indicates that at the initial stage, goethite is transformed to protohaematite at this temperature. When the same disk is then heated at 500 °C during one day (24 h), a different spectrum results, presumably corresponding to a transitional oxide (Fig. 1b). This spectrum does not resemble a spectrum of magnetite<sup>3</sup> nor that of wustite<sup>11</sup>. On further heating of the disk at 500 °C this transitional oxide is transformed to maghaemite, as inferred from the corresponding IR spectrum (Fig. 1c). Additional evidence for this transformation is that the disk is attracted by a magnet, whereas disks of goethite in CsI or of pure CsI do not show magnetic activity in the presence of the same magnet.

Unsuccessful attempts were made to obtain maghaemite: (1) from more concentrated CsI disks of goethite; (2) from disks which were not reground and repressed for several times even after long grinding periods (up to 30 min); (3) from powders resulting after the third repressing and fourth regrounding of the disks, when the powders were consecutively heated to 250 and 500 °C; (4) if the temperature of the second stage heating was below 500 °C. In (1), (2) and (3) protohaematite was the main product of the thermal treatment; in (4) the transitional oxide was the main product at >400 °C.



**Fig. 1** IR spectra of iron oxides in CsI disks: *a*, protohaematite obtained by heating goethite disk one day at 250 °C; *b*, transition iron oxide obtained by heating disk *a* 1 d at 500 °C; *c*, maghaemite obtained by heating disk *a* 4 d at 500 °C; *d*, protohaematite obtained by heating goethite powder 5 d at 450 °C; *e*, transition iron oxide obtained by heating disk *d* 1 d at 500 °C; *f* maghaemite obtained by heating disk *d* 14 d at 500 °C. (*f* also contains some of the transition iron oxide; *e* and *f* contain small amounts of protohaematite).

The transformation of protohaematite (obtained by heating goethite powder at 450 °C for 5 days) to maghaemite in CsI disks was also followed by IR spectroscopy (Fig. 1*d, e, f*). Here again, the optimal conditions for obtaining maghaemite are a small oxide concentration, regrinding and re-pressing the disk for at least three times and heating the disk to 500 °C as a whole and not as powder.

As with goethite the transformations of protohaematite into maghaemite in CsI disk also occurs through a transitional oxide. However, bigger amounts of protohaematite remain untransformed after one day heating at 500 °C, compared with the amounts of protohaematite which are found in the goethite disk heated in the same conditions. Moreover, when protohaematite is the starting material the reaction rates are slower. The reaction was completed after less than four days when starting with goethite, but was far from completion even after 14 days when starting with protohaematite (compare Fig. 1*c* and *f*).

The thermal transformations of haematite (obtained by heating goethite powder at 1,000 °C) and of natural Al-bearing goethite (from Venezuelan laterites<sup>12</sup>) to maghaemite in CsI disks were unsuccessful in the present experimental conditions.

The protohaematite → maghaemite transformation is an epitaxial process in which accord between the initial and resultant lattices occurs in two dimensions. Protohaematite has a hexagonal close-packed oxygen sublattice whereas maghaemite has a cubic one. The ordering of layers shifts from *ababab* in protohaematite to *abcabc* in maghaemite. One would expect that such a shift might occur when the *ababab* association is highly defective and/or the crystal particles are very small, as in the case of protohaematite but not of haematite.

The crystal defects, the degree of crystallinity and the small size of the protohaematite particles cause the X-ray diffraction

peaks to broaden. Protohaematite was prepared by heating gradually to 450 °C a disk composed of 150 mg synthetic goethite and 300 mg CsI (the mixture being ground for 30 min before pressing). Table 1 summarises widths of half heights of the characteristic diffraction peaks showing that there is an inverse correlation between the widths of X-ray diffraction peaks of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> and the ease of forming maghaemite. As may be expected from thermodynamic considerations haematite with the highest degree of crystallinity fails to transform into maghaemite, whereas protohaematite formed in a CsI disk and which has the lowest degree of crystallinity, is most easily transformed into maghaemite.

The present study does not enable us to draw a firm conclusion on the stage at which the crystal nuclei of maghaemite are obtained. However, we can suggest that the nucleation of maghaemite occurs in the CsI/Fe<sub>2</sub>O<sub>3</sub> interface because: small particle-size is not sufficient for the reaction to occur; the importance of regrinding and re-pressing; the reaction does not take place in a powdered medium; and other alkali halides (including KI) fail to cause maghaemite to form from goethite or from protohaematite with the same treatments.

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## Sequencing of the 3'-terminal region of a 16S rRNA gene from *Zea mays* chloroplast reveals homology with *E. coli* 16S rRNA

CHLOROPLASTS are semi-autonomous organelles which possess their own DNA coding for organelle-specific RNAs and proteins. The translation process within a chloroplast is mediated by its own ribosomes of the 70S type, which are different from the 80S ribosomes of the cytoplasm. In view of this key function of 70S ribosomes for the organelle-specific protein synthesis we have undertaken to analyse rDNA from *Zea mays* chloroplasts at the nucleotide level, as we considered that this should allow a deduction of rRNA primary structures, and analysis of their mode of function and evolutionary relationship. DNA from *Zea mays* chloroplasts is a circular molecule of about 135 kilobase pairs, on which two rRNA coding regions are positioned in opposite orientation within two 22-kilobase pair



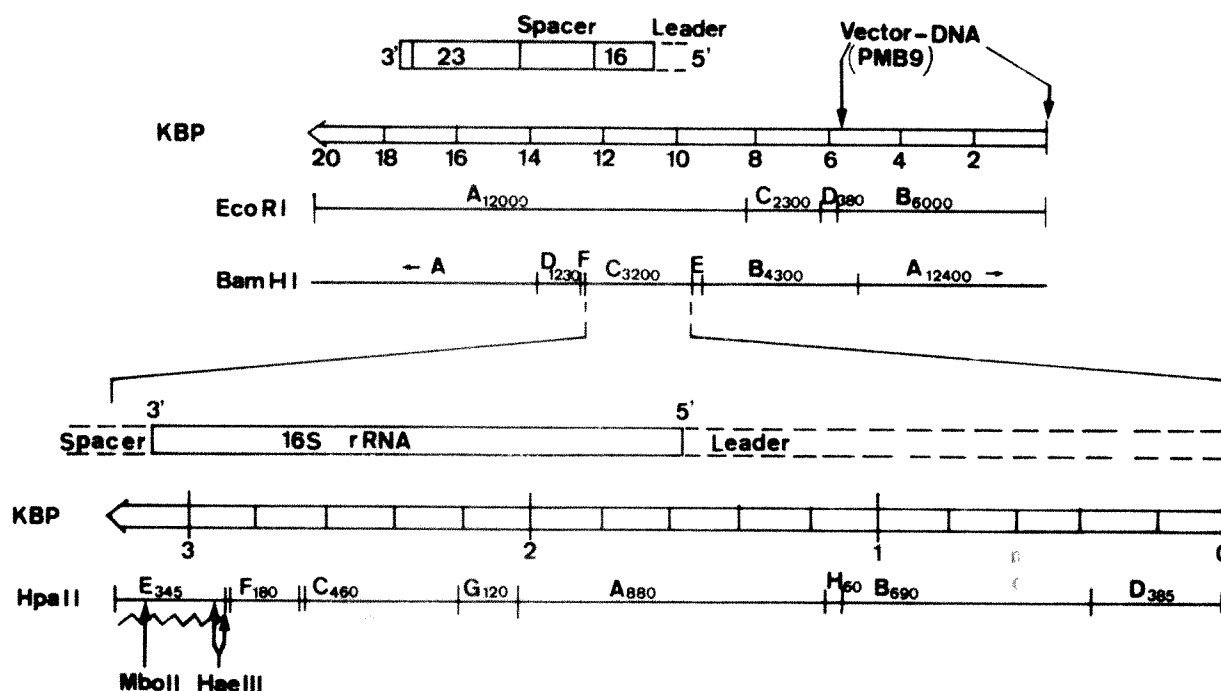


Fig. 1 Position of rRNA genes and of fragment *Bam*HI·C/*Hpa*II·E<sub>345</sub> (wavy line) on the physical map of plasmid pZmc134. Subscript numbers of the fragments refer to their approximate size in base pairs.

inverted repeats<sup>1</sup>. Each region includes one copy of a 16S rRNA, a 23S rRNA and a 5S rRNA gene and a spacer between the 16S and 23S rRNA genes. One such rDNA region has been linked to the plasmid vector pMB9 within the *Escherichia coli* clone pZmc134 (ref. 1), and this clone was therefore used for isolation, mapping and sequencing of the respective DNA fragments (Fig. 1)<sup>2</sup>. We present here the sequence coding for the 3'-terminal part of chloroplast 16S rRNA and for the adjacent part of the spacer region, and report that there is extensive homology between this region of 16S rRNA and the 16S rRNA gene from *E. coli*.

Hybridisation<sup>1,2</sup> and fine mapping<sup>2</sup> data indicated that the region coding for the 3' terminus of plastid 16S rRNA is likely to be positioned at one end of fragment *Bam*I·C, which, after further cleavage with the restriction enzyme *Hpa*II, produces the subfragment *Bam*I·C/*Hpa*II·E<sub>345</sub> (Fig. 1). This fragment, characterised by a *Bam*I site at one end and a *Hpa*II site at the other end (Fig. 2), was isolated and used for sequence analysis according to the dimethylsulphate/hydrazine procedure<sup>3</sup> with minor modifications as described<sup>4</sup>. After terminal labelling of this fragment the two labelled ends were separated by cleavage with *Hae*III or *Mbo*II (for position of cleavage sites see Figs 1,

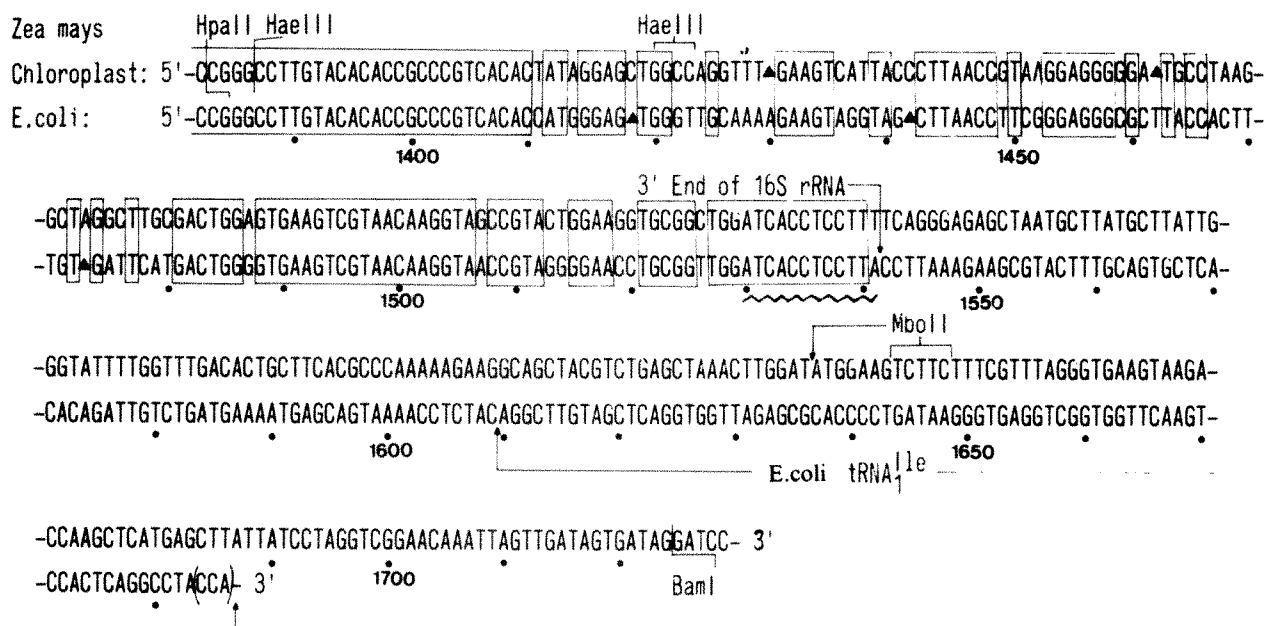


Fig. 2 3'-terminal portions of 16S rRNA genes from *Zea mays* chloroplasts (top row in each line, identical to the nucleotide sequence of fragment *Bam*I·C/*Hpa*II·E<sub>345</sub>) and from *E. coli*<sup>5,6,9,17</sup> (bottom row in each line). The sequences shown are of only one DNA strand, which is the non-coding, RNA-like strand. It should be noted that the sequence of the maize fragment *Bam*I·C/*Hpa*II·E<sub>345</sub> is depicted in opposite polarity to that in Fig. 1. Numbering refers to *E. coli* 16S rDNA<sup>5,9</sup>. The *E. coli* spacer part containing the tRNA<sup>ile</sup> gene is deduced from the tRNA<sup>ile</sup> primary structure<sup>17</sup>. Homologous sequences are marked by boxes. The sequences at the 3' end complementary to initiator regions of prokaryotic mRNAs<sup>7,10,11</sup> are underlined by wavy lines. Triangles within sequences symbolise deleted positions compared with the other DNA.





## Accumulation of an mRNA and protein in interferon-treated Ehrlich ascites tumour cells

INTERFERONS are glycoproteins which are synthesised by various vertebrate cells in response to virus infection or some other stimuli. They are secreted and then interact with other cells and convert these into an antiviral state, in which the multiplication of viruses is impaired<sup>1</sup>. It is thought that transcription and translation are required for the establishment of the antiviral state as actinomycin D or inhibitors of protein synthesis, suitably administered, prevent or delay the effect<sup>2,3</sup>. Results with enucleated cells also support this idea<sup>4</sup>. We show here that treatment of Ehrlich ascites tumour (EAT) cells with highly purified interferon results in the accumulation of a particular mRNA and the corresponding protein within the cells. However, we have not yet identified the function of the induced protein.

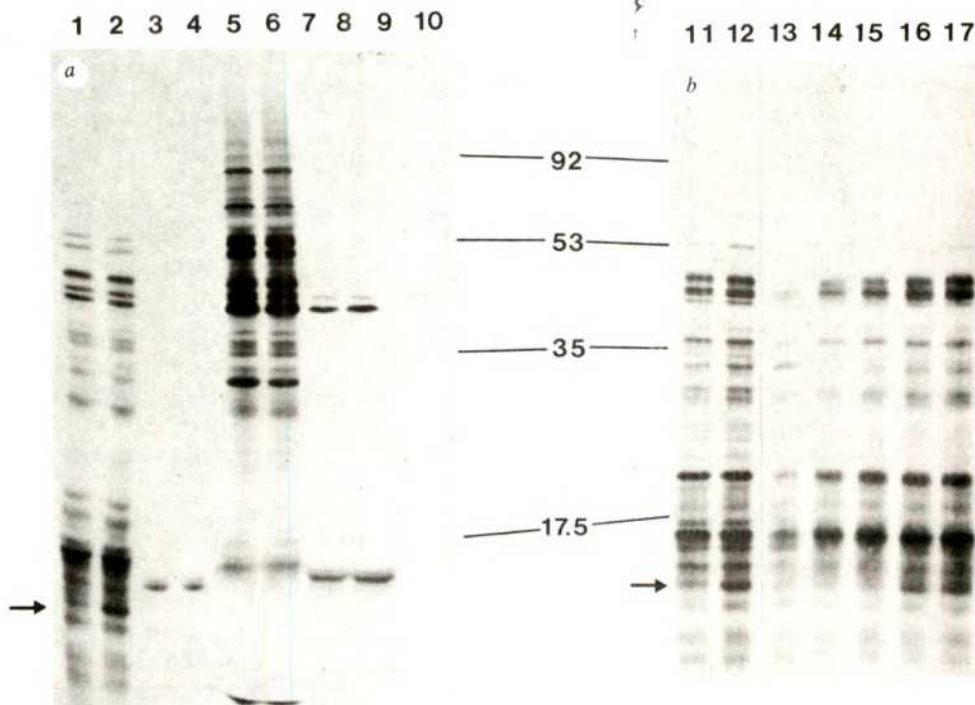
Messenger RNA was prepared from monolayers of EAT cells treated with 500 U ml<sup>-1</sup> interferon for 8 h and from control cells and was translated both in the wheat germ system and in the nuclease-treated reticulocyte lysate. The radioactive translation products were analysed by SDS gel electrophoresis (Fig. 1). In the wheat germ translation system (Fig. 1, tracks 1, 2), compared with the mRNA from control cells, the mRNA from interferon-treated cells yields an increased amount of one protein. This protein (Fig. 1, arrow) migrates with a molecular weight of

14,500. Translation of mRNA from cells which have been simultaneously treated with interferon and actinomycin D results in only the control level of the 14,500-MW protein (Fig. 1, track 13). The translation products in the reticulocyte lysate (Fig. 1, tracks 5, 6) extend to a higher molecular weight than those in the wheat germ system but the 14,500-MW region of the gel is obscured by the large amount of non-radioactive globin in the reticulocyte lysate.

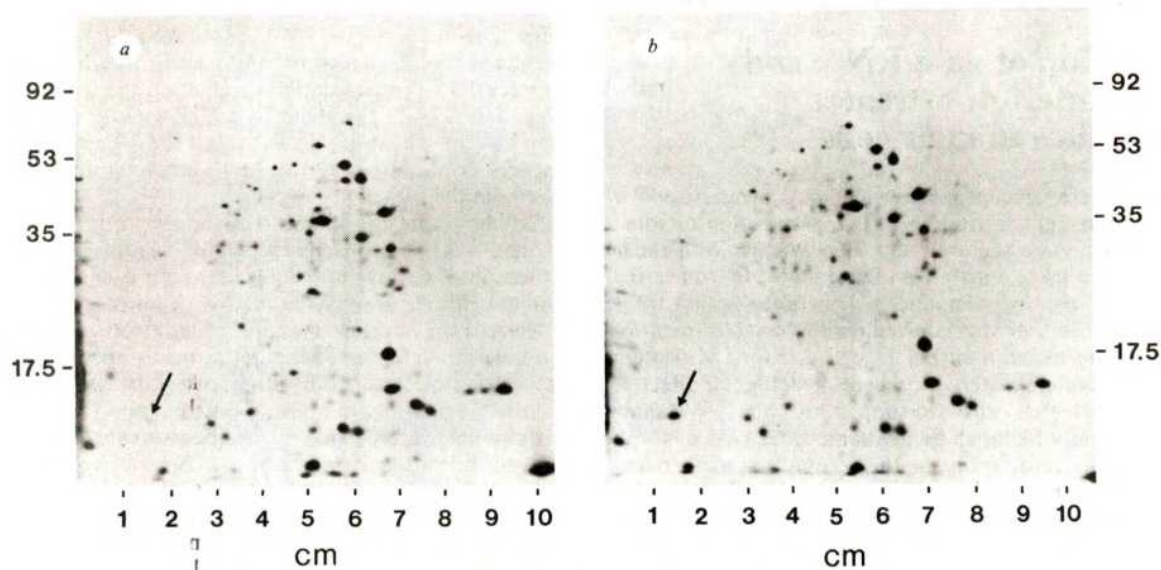
To show that the increased production of the 14,500-MW protein was not a consequence of differential post-translational processing, we first translated in the presence of radioactive amino acids separately the mRNA from control cells and the mRNA from interferon-treated cells. Each of these two mixtures was then further incubated with the products obtained by translating mRNA from either control or interferon-treated cells in the presence of non-radioactive amino acids. The second incubations were carried out in the presence of anisomycin to prevent further protein synthesis. There is neither degradation of the radioactive 14,500-MW protein when it is mixed with the non-radioactive translation products of control mRNA (Fig. 1, track 16), nor production of the 14,500-MW protein when the radioactive translation products of control mRNA are mixed with the non-radioactive translation products of the mRNA from the interferon-treated cells (Fig. 1, track 15). These results make it highly likely that treatment of the cells with interferon results in increased accumulation of the mRNA coding for the 14,500-MW protein.

To compare the *in vitro* translation products of mRNA from control cells and mRNA from interferon-treated cells more closely, we analysed them by two dimensional (2D) gel

**Fig. 1** Mouse EAT cells were grown in monolayers in GIBCO F15 medium containing 7.5% fetal calf serum. Mouse interferon ( $0.5-1 \times 10^9$  U mg<sup>-1</sup>) was prepared from EAT cells essentially as described previously<sup>11</sup>. Monolayers of EAT cells were treated as indicated with 500 U ml<sup>-1</sup> interferon for 7.5 h, or with 500 U ml<sup>-1</sup> interferon and 2  $\mu$ g ml<sup>-1</sup> actinomycin D for 7.5 h or kept as controls and then collected into phosphate buffered saline (PBS, 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.5 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.2) by scraping with a rubber policeman. The cells were washed twice with PBS, frozen, thawed and extracted twice with 0.15 M NaCl, 10 mM Tris-Cl pH 7.5, 1 mM MgCl<sub>2</sub>, 0.075% Triton X-100. After digestion with 0.3 mg ml<sup>-1</sup> proteinase-K in the presence of 0.5% SDS and 10 mM EDTA at 37 °C for 10 min, total cytoplasmic RNA was extracted by the phenol/chloroform/isoamyl alcohol method<sup>12</sup>. This RNA was fractionated on columns of oligo(dT)-cellulose (Collaborative T2) by application in 0.5 M NaCl, 10 mM Tris-Cl pH 7.5, 0.1% SDS and elution with 10 mM Tris-Cl pH 7.5, 0.1% SDS<sup>13</sup>. After recovery by ethanol precipitation, the unbound [poly(A)]<sup>-</sup> and the bound [poly(A)]<sup>+</sup> fractions were dissolved in water and stored at -70 °C. Eight 150 mm tissue culture dishes of 50% confluent control and interferon-treated cells typically yielded 2.05 mg poly(A)<sup>-</sup> RNA and 0.12 mg poly(A)<sup>+</sup> RNA. A similar quantity of cells treated with interferon and actinomycin D gave 1.57 mg poly(A)<sup>-</sup> RNA and 0.04 mg poly(A)<sup>+</sup> RNA. *In vitro* translations were in the wheat germ system<sup>14</sup> or mRNA-dependent reticulocyte lysate<sup>6</sup> with 150  $\mu$ Ci ml<sup>-1</sup> of a mixture of <sup>35</sup>S-methionine and cysteine as radioactive label. Poly(A)<sup>-</sup> RNA was translated at 500  $\mu$ g ml<sup>-1</sup> and poly(A)<sup>+</sup> RNA at 60  $\mu$ g ml<sup>-1</sup> except in track 13 where poly(A)<sup>+</sup> RNA was at 20  $\mu$ g ml<sup>-1</sup>, reflecting the lower amount of RNA in the actinomycin D-treated cells. After translation at 30 °C for 60 min, aliquots were analysed by SDS gel electrophoresis<sup>6</sup> and fluorographs<sup>15,16</sup> are shown. *a*, Tracks 1, 2, 5, 6 poly(A)<sup>-</sup> RNA, 3, 4, 7, 8 poly(A)<sup>+</sup> RNA, 9, 10 no mRNA was translated in 1, 2, 3, 4, 9 the wheat germ system or 5, 6, 7, 8, 10 the reticulocyte lysate. Tracks 1, 3, 5, 7 are RNA from control cells, 2, 4, 6, 8 RNA from interferon-treated cells. *b*, Translation in wheat germ system of poly(A)<sup>+</sup> RNA from control cells (11), interferon-treated cells (12), cells treated with interferon and actinomycin D (13). Tracks 14-17: poly(A)<sup>+</sup> RNA from control or interferon-treated cells was translated for 60 min in the wheat germ system in the presence of <sup>35</sup>S-methionine and cysteine and then an aliquot of each was mixed with an equal volume of duplicate translations containing either control or interferon-treated cell poly(A)<sup>+</sup> RNA and no radioactive label. These reactions were then further incubated at 30 °C for 45 min in the presence of 0.2 mM anisomycin. Tracks 14, 15 first (labelled) incubation control cell mRNA; tracks 16, 17 first (labelled) incubation interferon treated mRNA; tracks 14, 16 mixed with control cell mRNA translation products; tracks 15, 17 mixed with interferon-treated cell mRNA translation products. The experiments in (*b*) used independent preparations of mRNAs and wheat germ system from those in (*a*). The vertical scale shows the positions to which standard proteins (molecular weights in kilodaltons) migrated on the gel.



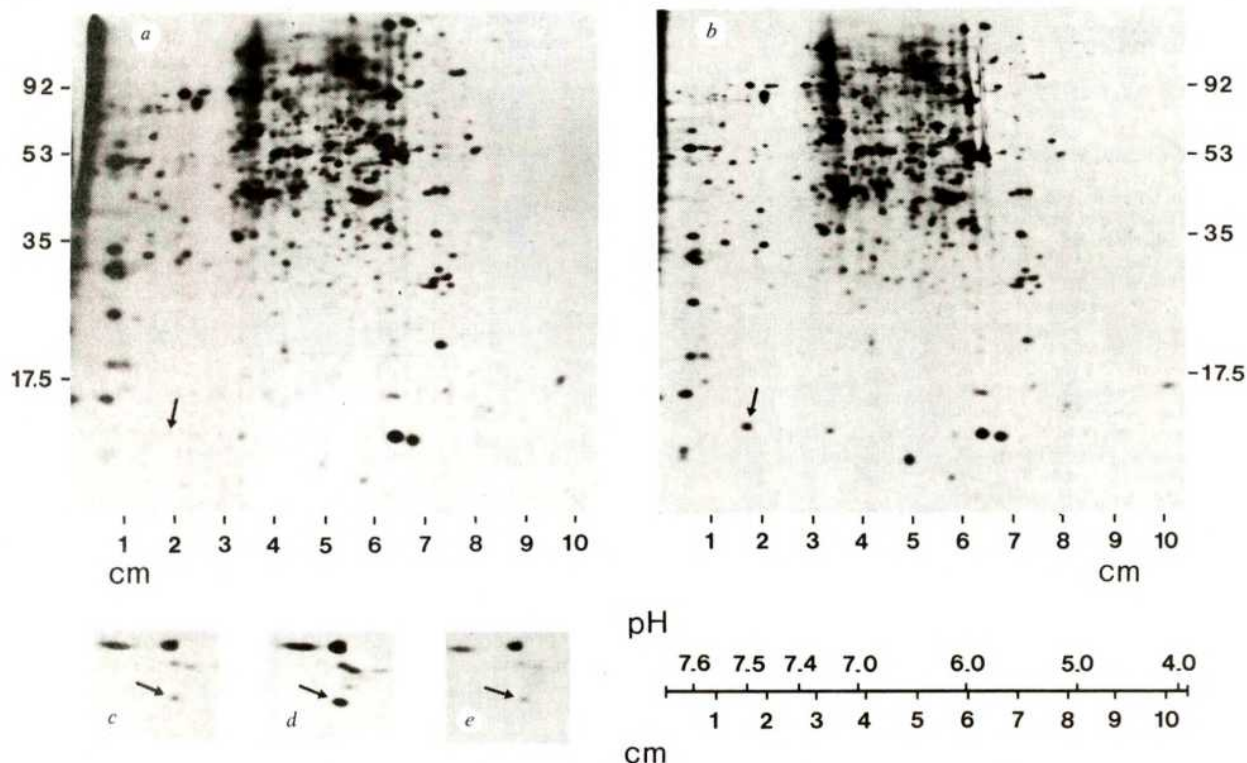




**Fig. 2** Poly(A)<sup>+</sup> RNA from *a*, control cells; *b*, cells treated with 500 U ml<sup>-1</sup> interferon for 8 h was translated at 60 µg ml<sup>-1</sup> for 60 min in the wheat germ system with <sup>35</sup>S-methionine and cysteine as radioactive label. Samples were then made up to 9 M urea, 2% ampholines (pH 3–10, Bio-Rad), 1% NP-40, 2.5% 2-mercaptoethanol and analysed by 2D gel electrophoresis as described by O'Farrell<sup>5</sup> except that the isoelectric focusing gels contained 2% Bio-Rad ampholines (pH 3–10) and the second dimension was on non-gradient 15% gels. The figure shows fluorographs of the gels. The equivalence of cm and pH is given in Fig. 3 and the vertical scale shows the positions to which standard proteins (molecular weights given in kilodaltons) migrated on the gel.

electrophoresis<sup>5</sup> (Fig. 2). In this system the 14,500-MW induced protein (arrowed) shows up clearly at pI 7.5. We cannot yet find any other significant difference between the *in vitro* translation products of the two mRNA preparations.

The experiments shown in Fig. 3 were designed to find whether the elevated level of mRNA coding for the 14,500-MW protein is manifested in an increased amount of this protein in interferon-treated cells. EAT cells were treated with interferon



**Fig. 3** EAT cells were treated with interferon and actinomycin D as below and then labelled with 60 µCi ml<sup>-1</sup> of a mixture of <sup>35</sup>S-methionine and cysteine for 1 h. At the end of the labelling the medium was removed, the monolayer was washed with ice-cold PBS and the cells were lysed in 0.4 ml of 9 M urea, 2% NP-40 per 60-mm dish. The viscous lysate was sonicated for about 15 s to disperse the DNA. Samples of 10 µl of the lysate were made up to 9 M urea, 2% ampholines (pH 3–10, Bio-Rad), 1% NP-40, 2.5% 2-mercaptoethanol and analysed by 2D gel electrophoresis as in Fig. 2 except that in *c*, *d* and *e* non-gradient 12.5% gels were used for the second dimension. Radioactive proteins were detected by fluorography. *a*, *c*, Control cells; *b*, *d*, cells treated with 500 U ml<sup>-1</sup> interferon for 7.5 h. *e*, cells treated with 500 U ml<sup>-1</sup> interferon and 2 µg ml<sup>-1</sup> actinomycin D for 7.5 h. The pH gradient in the isoelectric focusing gel was measured with a Bio-Rad propHiler and the equivalence of pH and the cm scale is shown. The shallow pH gradient at the basic end of the gel has been noted previously by O'Farrell<sup>17</sup>. Parts *a*, *b* were from the same experiment as Fig. 2, and *c*, *d* and *e* from another. In *c*, *d* and *e* only the region of the gel containing the induced protein is shown. The vertical scale in *a*, *b* shows the positions to which standard proteins (molecular weights in kilodaltons) migrated on the gel.



(500 U ml<sup>-1</sup>) for 7.5 h and then incubated with <sup>35</sup>S-labelled amino acids for 1 h. The labelled proteins from the cells were analysed by 2D gel electrophoresis and the pattern of radioactive proteins was compared with that derived from similarly labelled control cells (Fig. 3a, b). The interferon-treated cells show strongly increased labelling of a protein which has the same mobility as the protein observed in Fig. 2 to be present in increased amounts in the translation products of the mRNA from interferon-treated cells. There are other proteins on the 2D gels whose labelling sometimes seems to be affected *in vivo* by interferon treatment but we have not fully established conditions for the changes in labelling of these proteins or found any changes in the corresponding mRNA levels. If cells are treated as above with interferon (Fig. 3d) except that actinomycin D is added at the same time as the interferon (Fig. 3e), the labelling of the 14,500-MW protein is the same as in the control cells (Fig. 3c). When cells are treated with interferon for various lengths of time then incubated with radioactive amino acids and analysed as above on 2D gels, the induction can just be observed after 5 h of interferon treatment and seems to be maximal by 9 h of interferon treatment (data not shown).

We scanned the spots on the fluorograms of the 2D gels and compared the relative blackening on the films to obtain an approximate estimate of the degree of induction of the 14,500-MW protein. This results in values of 7.5-fold for the labelled cells and five-fold for the *in vitro* translations.

We have not yet been able to equate this induced protein with any of the enzymes known to increase in activity after interferon treatment. We have not found any difference in double-stranded RNA-dependent protein kinase activity<sup>6-8</sup> in the translation products of the mRNA from control and interferon-treated cells (data not shown). Nor do we detect any difference in the ability of the translation products to synthesise the 2,5A class of compounds<sup>9</sup>. The 14,500-MW protein does not appear to be mouse globin (determined by co-electrophoresis) or interferon (assayed by antiviral activity). As 500 U ml<sup>-1</sup> interferon slows the growth of these cells by less than 5% in the first 5 days of treatment, it seems unlikely that the induction we have observed is an indirect consequence of an inhibition of cell growth by interferon. We presume that our induced protein is different from a 56,000-MW protein recently reported by Ball<sup>10</sup> to be induced in chick cells by interferon and we are currently trying to establish the function of the induced 14,500-MW protein and to examine other cell types for similar phenomena.

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## Hydroxylamine stimulates carboxylase activity and inhibits oxygenase activity of cyanobacterial RuBP carboxylase/oxygenase

RIBULOSE-1,5-BISPHOSPHATE (RuBP) carboxylase/oxygenase (EC 4.1.1.39) catalyses both the photofixation of CO<sub>2</sub> and light-dependent O<sub>2</sub> uptake (photorespiration) in photo-trophs<sup>1-4</sup>. Photorespiration is generally considered to be a wasteful process, reducing net plant productivity<sup>5,6</sup> and N<sub>2</sub> fixation where it occurs<sup>7</sup>. The selective inhibition of RuBP oxygenase activity, either by endogenous metabolites or exogenous effectors, while allowing carboxylation to continue, has been viewed as a means of increasing plant productivity<sup>5,6</sup>. There is no previous unequivocal evidence of one metabolite differentially affecting both reactions. Here, we present data on how it is possible, *in vitro*, to stimulate carboxylase activity and inhibit oxygenase activity simultaneously in extracts of the cyanobacterium *Anabaena cylindrica* by adding hydroxylamine, an intermediate in the reduction of nitrate to ammonia in cyanobacteria<sup>8</sup> and higher plants<sup>9</sup>.

*Anabaena cylindrica* Lemm. (1403/2a) (Culture Centre of Algae and Protozoa, Cambridge, UK) was grown in pure culture as described previously<sup>10</sup>. RuBP carboxylase activity, measured as RuBP-dependent <sup>14</sup>CO<sub>2</sub> incorporation into acid-stable material, and RuBP oxygenase activity, assayed as RuBP-dependent O<sub>2</sub> consumption, were followed simultaneously in an O<sub>2</sub> electrode. Preincubation for 10 min at between 0 and 40 °C had no effect on either RuBP carboxylase or oxygenase activities subsequently assayed at 30 °C. However, a 60% increase in both activities of the enzyme occurred after incubation at 50 °C for 10 min, before assay at 30 °C. The ratio of oxygenase/carboxylase activity remained fairly constant at around 0.063, irrespective of the temperature within the range 0-50 °C. This is the first evidence of heat activation of the enzyme from a cyanobacterium and accords with the finding of heat activation of the eukaryotic enzyme<sup>11-14</sup>. Experiments detailed below were carried out after pretreatment of the *A. cylindrica* enzyme at 50 °C for 10 min, followed by equilibration at 30 °C for 2 min.

**Table 1** Effects of various nitrogen compounds on the RuBP carboxylase/oxygenase activities of *Anabaena cylindrica*

Nitrogen compound added	RuBP carboxylase		RuBP oxygenase	
	1.25 mM*	10 mM*	1.25 mM*	10 mM*
None	100	100	100	100
KNO <sub>3</sub>	102	101	93	86
KNO <sub>2</sub>	82	88	72	79
NH <sub>2</sub> OH	NT	171	NT	72
NH <sub>4</sub> Cl	94	101	89	95

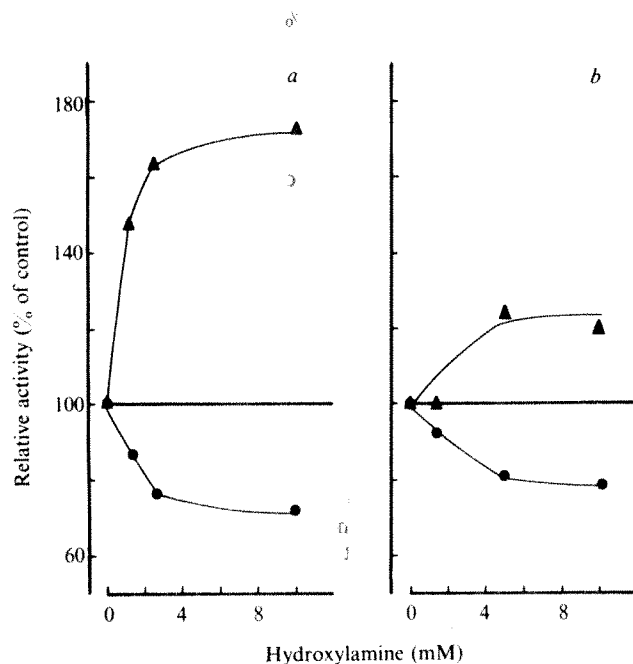
The preincubation temperature was 50 °C and the assays were carried out in the presence of 0.14 mM RuBP; other experimental details as in legend to Fig. 1. Activities are expressed as a percentage of those measured in the absence of nitrogen compounds.

\* Final concentration of added nitrogen compound; NT, not tested.

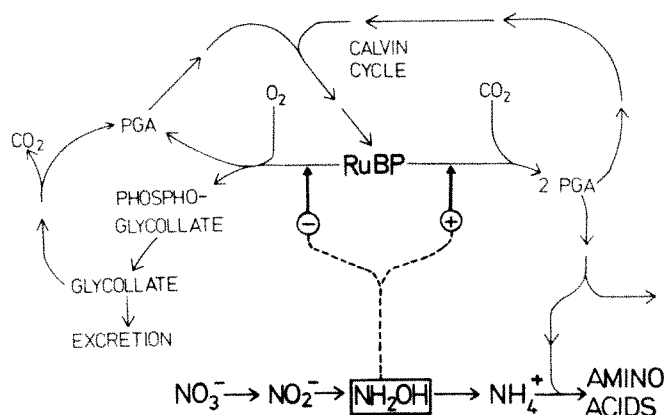
Data on the effect of various nitrogen compounds on RuBP carboxylase/oxygenase activities (Table 1) show that of the compounds tested only hydroxylamine had any significant effect, stimulating carboxylase activity markedly and inhibiting oxygenase activity. Figure 1 shows the differential effects of hydroxylamine on *A. cylindrica* RuBP carboxylase and oxygenase activities at various effector concentrations. RuBP oxygenase was again inhibited by hydroxylamine, while carboxylase

was simultaneously enhanced when the hydroxylamine concentration was increased up to 10 mM. The differential effect of hydroxylamine on these reactions was greater in the presence of 0.14 mM RuBP than at 0.28 mM RuBP.

Our findings with hydroxylamine show similarities to and differences from the results of Bhagwat *et al.*<sup>15</sup> using the spinach enzyme. Like them, we found an inhibition of RuBP oxygenase activity by hydroxylamine, but also, unlike them, we found a stimulation of RuBP carboxylase activity by hydroxylamine. Although this may reflect a difference between the prokaryotic and higher plant enzyme, it could also be due to the assay methods used. In particular, the Indian workers<sup>15</sup> used separate



**Fig. 1** Effects of increasing hydroxylamine concentrations on *Anabaena cylindrica* RuBP carboxylase/oxygenase activities at two RuBP concentrations. 8 l of log phase culture were collected by centrifugation, washed and resuspended in 2 vol. buffer (20 mM Tris, 1 mM Na<sub>2</sub>EDTA, 10 mM MgCl<sub>2</sub>, 50 mM NaHCO<sub>3</sub>, 0.5 mM dithiothreitol and 1 mM phenylmethylsulphonyl fluoride, pH 8.0). Cells were disrupted by French pressure cell treatment at 110 MPa at 4 °C. The extract was then centrifuged at 15,000g for 20 min and the supernatant re-centrifuged at 100,000g for 60 min. After (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> precipitation of the supernatant, the 25–45% fraction was resuspended in buffer and concentrated by dialysis against buffer. This served as the enzyme preparation. Heat activation was carried out on 125-μl samples of enzyme in 1.5-ml capped vials to prevent loss of CO<sub>2</sub>. The vials were incubated at 50 °C for 10 min, then maintained at 30 °C, the assay temperature, for 2 min before assay. RuBP carboxylase and oxygenase activities were measured simultaneously using a Pt-Ag oxygen electrode (Rank). Assays were started by adding 50 μl enzyme (10–40 μg protein) to the reaction mixture containing 100 mM Bicine-NaOH buffer, pH 8.3 (equilibrated with CO<sub>2</sub>-free air), 0.27 mM NaH<sup>14</sup>CO<sub>3</sub>, 20 mM MgCl<sub>2</sub> and 0.14 mM or 0.28 mM RuBP. In the final assay concentrations produced (21% O<sub>2</sub>, 2.56 mM NaHCO<sub>3</sub> at pH 8.3) carboxylase and oxygenase activities were simultaneously followed. Oxygen uptake was linear for at least the first 100 s and data presented are those obtained over this 100-s period. Carboxylase activity was determined, after 100 s, by removing a 100-μl aliquot of the reaction mixture into trichloroacetic acid (final concentration 40% w/v). <sup>14</sup>C incorporated into acid-stable material was then counted. Controls without RuBP were included. *a*, 0.14 mM RuBP; *b*, 0.28 mM RuBP; ▲, RuBP carboxylase; ●, RuBP oxygenase. Activities are expressed as a percentage of those measured minus hydroxylamine. Specific activities, measured minus hydroxylamine were: RuBP carboxylase, 8.13 and 12.13 μmol CO<sub>2</sub> fixed per mg protein per h at 0.14 and 0.28 mM RuBP respectively, and RuBP oxygenase, 0.88 and 0.90 μmol O<sub>2</sub> consumed per mg protein per h at 0.14 and 0.28 mM RuBP respectively.



**Fig. 2** Possible interactions between hydroxylamine and RuBP carboxylase/oxygenase based on our *in vitro* data. Solid lines represent metabolic pathways, broken lines represent inhibition (–) or activation (+) of enzymatic activities. PGA, 3-phosphoglyceric acid.

RuBP carboxylase and oxygenase assays and saturating bicarbonate and O<sub>2</sub> concentrations, starting both reactions by the addition of RuBP. We measured RuBP carboxylase and oxygenase activities simultaneously in the same vessel using assays with sub-optimal concentrations of CO<sub>2</sub> and O<sub>2</sub> respectively, and started the reactions by the addition of heat-activated enzyme. The effect of hydroxylamine on RuBP carboxylase may depend on CO<sub>2</sub> concentration, as well as on RuBP concentration. It has also been suggested that high CO<sub>2</sub> concentrations may protect the spinach RuBP carboxylase<sup>15</sup> and our data support this contention.

The results point to a possible close coordination of nitrogen and carbon metabolism in *Anabaena*, as Solomonson and Spehar have postulated for *Chlorella*<sup>16</sup>. The latter workers suggest that hydroxylamine, produced during nitrate assimilation, combines with glyoxylate, produced by glycollate oxidation, to form glyoxylate oxime, and thence cyanide. Cyanide, produced in photorespiratory conditions, may thus effect the reversible inhibition of nitrate reductase at low concentrations. However, although cyanide production by *Chlorella vulgaris* has been demonstrated<sup>17</sup> and cyanide inhibits *Chlorella variegata* nitrate reductase *in vitro*<sup>18</sup>, this inhibition does not occur when nitrate is present (see Table 4 of ref. 18). The inhibition of RuBP oxygenase by hydroxylamine would serve to reduce photorespiratory glyoxylate production and, according to the model of Solomonson and Spehar<sup>16</sup>, cyanide production. Our data, showing the direct inhibition of RuBP oxygenase by hydroxylamine, suggest that a regulation of photorespiratory carbon flow and nitrate assimilation may occur without the involvement of cyanide.

*In vivo*, the provision of adequate carbon skeletons is essential to prevent uncoupling by high pools of ammonia (Y. Steinitz, G.A.C. and W.D.P.S. in preparation). Our data suggest that in the presence of nitrate, hydroxylamine may contribute to the fine coordinate control of ammonia levels and the supply of carbon skeletons, as outlined in Fig. 2. The stimulatory effect of hydroxylamine on RuBP carboxylase and its concomitant inhibition of photorespiration would increase net carbon gain, thus helping to ensure the adequate supply of carbon skeletons for amino acid biosynthesis. The *in vivo* significance of these findings will depend on the size of the intracellular pools of hydroxylamine, or if hydroxylamine remains enzyme bound<sup>19</sup>, on an association between RuBP carboxylase/oxygenase and nitrate/nitrite reductase(s), or both. There is no evidence yet for a close association of such enzymes in cyanobacteria but there is evidence for such an association in eukaryotic systems<sup>20–22</sup>.

It is also known that nitrate stimulates O<sub>2</sub> evolution when supplied to algae incubated in CO<sub>2</sub>-limited conditions and this

has been attributed to nitrate acting as an alternative electron acceptor to CO<sub>2</sub> (ref. 23). Our data suggest that if hydroxylamine inhibits RuBP oxygenase activity *in vivo* this would decrease O<sub>2</sub> uptake and be responsible, in part at least, for the enhancement of O<sub>2</sub> evolution.

Irrespective of their *in vivo* significance, our data establish the fact that the RuBP carboxylase/oxygenase from an oxygenic photosynthetic prokaryote can be differentially regulated by chemical means.

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## Nitrogen fixation in coral reef sponges with symbiotic cyanobacteria

NITROGEN FIXATION by endosymbiotic cyanobacteria (blue-green algae) results in an important input of nitrogen into terrestrial ecosystems<sup>1</sup>; however, there is as yet little information on its significance in the marine environment<sup>2,3</sup>. Symbiosis of cyanobacteria with marine animals, although rare, is known to occur in an echiuroid worm<sup>4</sup> and in sponges from coral reefs<sup>5</sup> and the Mediterranean<sup>6</sup>. In the present study of sponge–cyanobacterial symbioses, several sponges from a coral reef in the Red Sea were tested, using the acetylene reduction technique<sup>7</sup>, immediately after their collection on reef-based platforms for their ability to fix nitrogen. Nitrogenase activity was detected in two sponges with cyanobacteria but not in a third with no cyanobacteria. This is the first demonstration of nitrogenase activity in an animal with symbiotic cyanobacteria. In two previous reports of nitrogen fixation in marine animals, the activity was attributed to bacteria in the gut<sup>8,9</sup>. Although these preliminary experiments are not sufficient to assess the significance of cyanobacterial nitrogenase activity in sponges, it is possible that any additional fixed nitrogen would be beneficial

to sponges in tropical waters which are low in available nitrogen<sup>10</sup> and in particulate nutrients<sup>11,12</sup>.

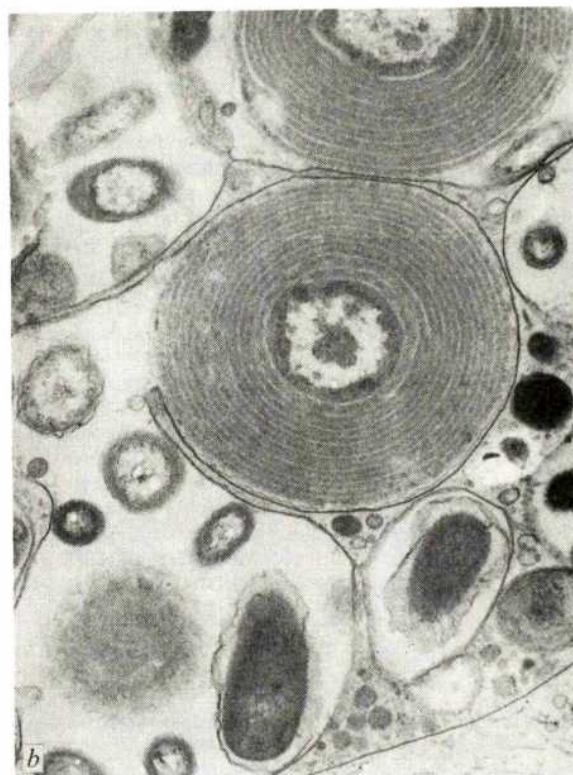
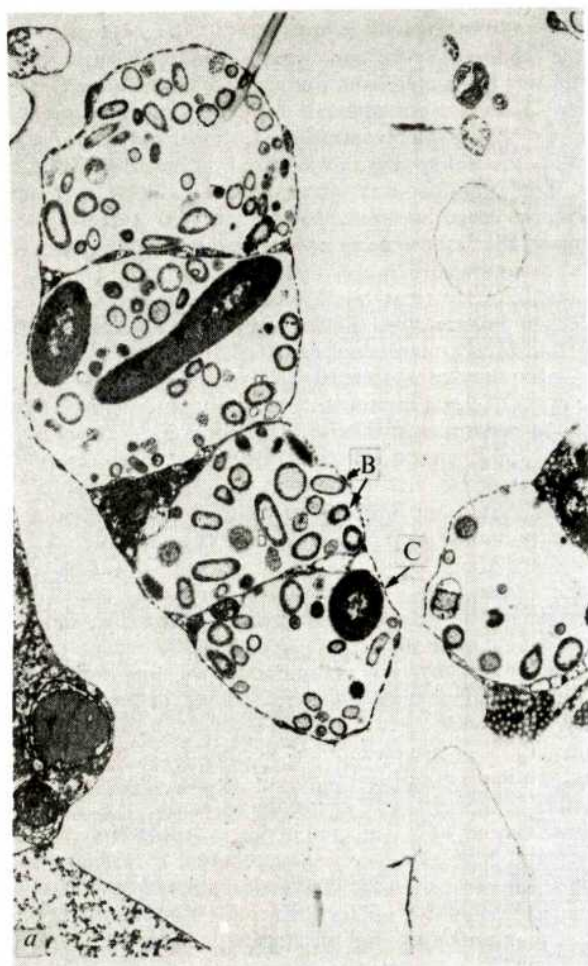
Three sponges, two containing cyanobacteria and one without, were collected from illuminated habitats at a depth of 6–7 m on Harvey Reef adjacent to Port Sudan. The consistent presence of unicellular cyanobacteria, similar to those observed in other coral reef sponges<sup>5</sup>, was confirmed by electron microscopy. The cyanobacteria were observed, along with other bacteria, in large vacuolated cells, termed bacteriocytes<sup>13</sup>, throughout the tissue of *Siphonochalina tabernacula* (Row) (Fig. 1). The cyanobacteria were present in low numbers and are estimated to occupy less than 1% of the sponge volume seen in the electron micrographs. Similar cyanobacteria were observed in the thin brown pigmented ectosome of *Theonella swinhoei* Gray where they constitute 10–15% of the ectosome volume. These cyanobacteria are mostly free in the intercellular matrix with some present in specialised amoebocytes, termed cyanocytes<sup>5</sup>. Cyanobacteria numbers decrease away from the ectosome and are not evident in the deeper part of the endosome. Symbiotic bacteria are rare in the ectosome and particularly numerous in the endosome. Large populations of bacteria are the only symbionts observed in *Inodes erecta* (Keller).

Nitrogenase activity was determined using the acetylene reduction test as modified by Flett *et al.*<sup>14</sup>, except that the acetylene gas phase was maintained over the liquid phase because the sponge tissue was too fragile to agitate. Tissue samples were suspended in filtered seawater and incubated in 100-ml glass syringes at a depth of 30 cm in seawater exposed to saturating illumination (full sunlight,  $5 \times 10^{16}$  quanta cm<sup>-2</sup> s<sup>-1</sup>) and at temperatures 1–4 °C above the ambient 31 °C. Duplicate 5-ml gas samples were collected in pre-evacuated tubes ('vacutainers') and estimated for ethylene content by gas chromatography at laboratories in the University of Bristol and Westfield College, London. After correcting for initial contamination, the ethylene concentration was determined using a variation of Henry's law<sup>14</sup>.

When similarly sized pieces of *S. tabernacula* and *I. erecta*, collected from similar habitats, were incubated with acetylene, ethylene was produced in *S. tabernacula* and apparently consumed in *I. erecta* (Fig. 2a). Adjacent pieces from a large *S. tabernacula* specimen were incubated in light or dark, with considerably more ethylene being produced in the light sample (Fig. 2b). The rate of production of ethylene in *S. tabernacula* was linear during the initial 2 h of incubation, with rates slightly decreasing in the subsequent period (Fig. 2c). In similarly sized tissue pieces of *S. tabernacula* incubated in the light with DCMU (dichlorophenyl dimethyl-urea), a specific inhibitor of photosystem II reaction having no effect on animal tissue, no appreciable reduction in total ethylene production was observed during the incubation period (Fig. 2c). The presence of DCMU had an apparently stimulatory effect on the O<sub>2</sub>-sensitive nitrogenase by depressing photosynthetic O<sub>2</sub> evolution, although this effect was probably compensated for later by the shortage of photosynthetically produced reductant<sup>15</sup>. In both dark and DCMU treatments, nitrogenase activity in the cyanobacteria may have persisted at the expense of preformed or non-photosynthetic source of reductant<sup>16</sup>, or activity could have been partly or fully bacterial in origin. In *T. swinhoei*, ethylene production in tissue samples containing between 20% and 30% pigmented ectosome was higher than in the non-pigmented endosome (Fig. 2d).

Nitrogenase activity is thought to be associated with the symbiotic cyanobacteria rather than other bacteria, for the following reasons: (1) nitrogenase activity was evident in *S. tabernacula* and *T. swinhoei*, which harbour symbiotic cyanobacteria, and was absent in *I. erecta*, which lacks cyanobacteria; (2) more ethylene was produced by the ectosome of *T. swinhoei*, which contained cyanobacteria, than by the endosome almost free of cyanobacteria; (3) nitrogenase activity was higher in illuminated tissue than in tissue incubated in the dark; and (4) the amounts of ethylene produced in *S. tabernacula* and





**Fig. 1** *a*, Electron micrograph showing cyanobacteria (C) and bacteria (B) within sponge cell vacuoles in the intercellular matrix of *Siphonochalina tabernacula*. Tissue was fixed in 2.5% glutaraldehyde in seawater:water (4:1), post-fixed in 1%  $\text{OsO}_4$  and embedded in Spurr's resin.  $\times 30,000$ . *b*, Section through sponge vacuole showing cyanobacterium with spiral thylakoid and adjacent smaller bacteria.  $\times 15,000$ .

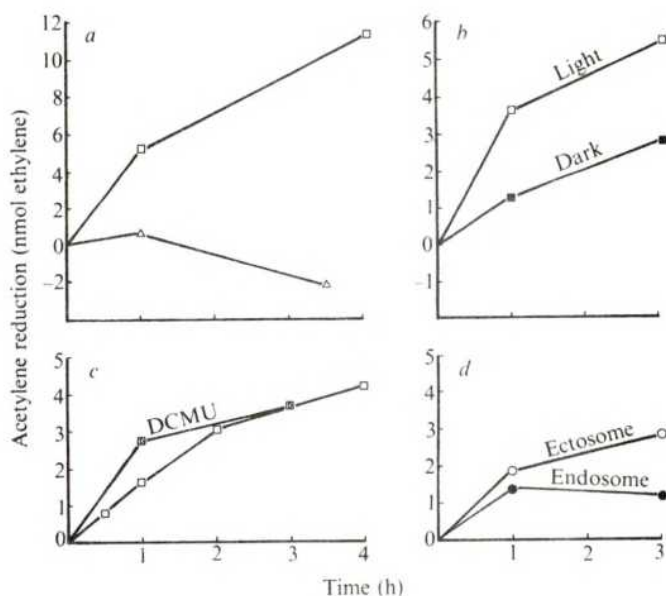
*T. swinhoei* were comparable although the bacterial population in *T. swinhoei* was many times larger than that in *S. tabernacula* and similar to that in *I. erecta*.

It is unlikely that nitrogenase activity was due to exogenous microorganisms colonising the surface, as the sponge epithelial cells (exo- and endopinacocytes) readily phagocytose particulate matter adhering to the surface<sup>12</sup>.

Although we conclude that the nitrogenase activity measured was due mainly or solely to symbiotic cyanobacteria, the role of other bacterial symbionts must be considered. Mixed populations of bacteria occur in sponges<sup>5,13</sup>, including phototrophic anaerobes<sup>17</sup>, and it is possible that some of these bacteria display nitrogenase activity. Associations between aquatic cyanobacteria and heterotrophic bacteria were considered to be beneficial to both partners<sup>18</sup>. Bacteria possibly enhance nitrogen fixation in freshwater cyanobacteria by creating a reducing atmosphere<sup>19</sup>. Synergistic nitrogen fixation may occur in sponges when cyanobacteria and heterotrophic bacteria are closely associated (Fig. 1).

More detailed experiments are required to establish the nutritional and ecological significance of the nitrogenase activity determined in these preliminary experiments. Although at first sight the levels of activity, 2–4 nmol  $\text{C}_2\text{H}_4$  produced per g (wet weight of sponge) per h for *S. tabernacula* and 3 nmol  $\text{C}_2\text{H}_4$  per g per h for *T. swinhoei*, seem to be rather low, it should be remembered that they originate from small populations of cyanobacteria in a tissue containing large quantities of intercellular matrix and inert skeleton. Comparisons with data obtained with other systems are not possible until the cyanobacterial population may be reliably estimated, and the cyanobacteria isolated and tested in pure culture.

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**Fig. 2** Acetylene reduction (nmol of ethylene in 5-ml gas samples): *a*, by equal (8.1 g) tissue samples of *Siphonochalina tabernacula* and *Inodes erecta* (no cyanobacteria) incubated in light; *b* during light and dark incubation of equal (3.4 g) samples of *S. tabernacula*; *c*, by equal (3.9 g) pieces of *S. tabernacula* incubated in the light in the presence or absence of  $10^{-5}$  M DCMU; *d*, by 2.1 g of tissue containing pigmented ectosome and 1.7 g of white endosome of *Theonella swinhoei*. Squares, *S. tabernacula*; triangles, *I. erecta*; circles, *T. swinhoei*.



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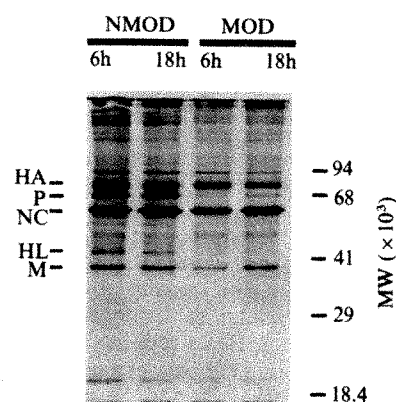
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## Antiviral antibody reacting on the plasma membrane alters measles virus expression inside the cell

SOME viruses are known to persist despite a functional antiviral immune response by the host. For example, measles or measles-like viruses can cause persistent infection in man leading to a progressive degenerative disease, subacute sclerosing panencephalitis (SSPE), characterised by unusually high titres of measles virus antibodies in serum and cerebral spinal fluids (see refs 1, 2 for reviews). Peripheral blood lymphocytes specifically responsive to measles virus antigens are generated and lyse target cells expressing virus antigens on their surfaces<sup>3,4</sup>. Sera from SSPE patients enhance rather than block or suppress immunologically mediated killing<sup>3–5</sup>. But although this anti-measles virus immune response is vigorous, the virus infection does not terminate because one can detect virus antigens and isolate infectious virus from central nervous system and lymphoid tissues<sup>1</sup>. To explain this we postulated that antibody to measles virus modulates or strips viral antigens off surfaces of virus infected cells<sup>6–7</sup>. This contention is based on the finding that specific measles antibody added to cultured infected cells modulate viral antigens on the cells' surfaces<sup>6</sup>. These cells then express less viral antigens on their surfaces and thereby avoid the host's immune assault. Cells denuded of viral antigens on their surfaces resist antibody and complement-mediated or immune lymphocyte-mediated killing<sup>6,7</sup> yet retain viral genetic information<sup>6</sup>. Further, during antibody modulation *in vitro*, the numbers of viral nucleocapsids accumulated inside the cell dramatically increase and are positioned randomly<sup>6</sup>. This *in vitro* picture closely resembles the distribution of nucleocapsids in cells obtained by biopsy from patients with SSPE<sup>1,8,9</sup>. Quantitatively, about 10- to 50-fold less antibody is needed on the surfaces of infected cells to modulate viral antigens than is needed to activate the complement system leading to immune lysis of infected cells<sup>5,10</sup>. To clarify the molecular events occurring during antibody-induced antigenic modulation, we have

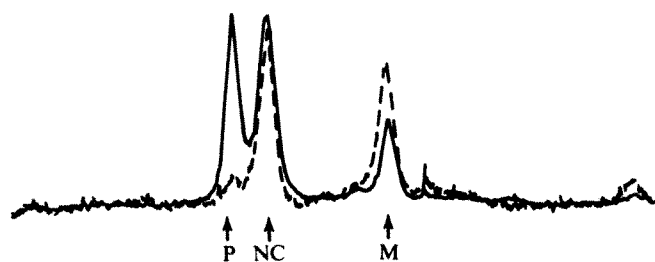
studied measles virus antibody bound to the surfaces of infected cells and ascertained what alterations occur within these cells. Here we report that measles virus antibodies added to virus infected HeLa cells decrease the content of both viral structural polypeptide haemolysin expressed on the cell surface and a structural polypeptide (measles virus phosphoprotein, P) found inside the cell. These observations indicate that antibody directed against an antigen on the cell surface can interfere with viral proteins expressed inside the cell. Because phosphoprotein seems to form complexes with the nucleocapsid, our findings may offer leads towards understanding viral regulation during persistence. We also note that three measles virus polypeptides—P, nucleocapsid (NC) and matrix protein (M)—are phosphorylated.

HeLa cells acutely infected with measles virus were cultured with and without antibody to measles virus in the media (see Fig. 1 for experimental details). The antibody used for modulation reacted with all measles virus structural proteins as determined by immune precipitation and analysis on polyacrylamide gel electrophoresis (PAGE). The antibody neutralised  $4.5 \times 10^5$  PFU per ml. After being cultured in antibody for 6 or 18 h<sup>6</sup>, HeLa cells were collected, washed four times in serum-free medium, and incubated in media free of either methionine or phosphorus. After 1 h the cells were washed and incubated in methionine- or phosphorus-free media to which <sup>35</sup>S-methionine or <sup>32</sup>P was added. Cells were collected 2 h later from both the controls (infected-unmodulated: NMOD) and the infected modulated (MOD) cultures, washed and lysed with 2% NP40 in TNE buffer. Nuclei and cell debris were removed by centrifugation and the cytosol preparations were incubated with



**Fig. 1** HeLa cells were infected in suspension with Edmonston strain of measles virus at a multiplicity of infection of 1. Cells were pelleted 24 h later and resuspended in Eagle's minimal essential media (MEM) containing either fetal calf serum (FCS) (non-modulated, NMOD) or antibodies to measles virus (modulated, MOD) for 1 h. The cells were pelleted and suspended in fresh MEM with 20% serum containing antibodies to measles virus or 20% FCS and placed on a rotating platform at 37 °C. At 6 h and 18 h, cells were collected by low-speed centrifugation, washed four times with serum-free MEM and incubated in methionine-free media. After 1 h in which  $10 \mu\text{Ci } ^{35}\text{S}$ -methionine per ml was added and the cells incubated at 37 °C, the cells were washed once with phosphate-buffered saline and lysed at 4 °C with NP40 in TNE buffer containing 1 mM PMSF. Nuclei and cell debris were removed 15 min later by centrifugation at 1,000g for 15 min, and the cytosols (300  $\mu\text{l}$ ) were precipitated with 10  $\mu\text{l}$  hyperimmune antibody to measles virus. Incubation lasted overnight at 4 °C. Thereafter, an excess of heat-inactivated, formalin-fixed *Staph. aureus* was added to precipitate all the formed complexes. Precipitates were washed twice in 0.5% NP40 in TNE buffer and twice again with 0.02% NP40 in buffer, suspended in sample preparation buffer containing 1% SDS and 1% 2-mercaptoethanol and immersed in boiling water for 2 min. The bacteria were pelleted at 1,000g for 15 min and supernatants removed. The immune precipitates were counted and applied to 10.5% SDS gels for PAGE analysis. Gels were stained, fixed and dried on filter paper. Kodak XRP-1 X-ray film was exposed to the dried gels and developed. Appropriate molecular weight markers were run in adjacent lanes.

Data shown are representative of seven such experiments.



**Fig. 2** HeLa cells infected with measles virus were treated as described in Fig. 1 except that phosphorous-free media and  $^{32}\text{P}$  ( $50 \mu\text{Ci ml}^{-1}$ ) replaced the  $^{35}\text{S}$ -methionine and cells were collected 12 h post-infection. The ordinate represent relative density and the abscissa the relative migration. The three phosphorylated measles virus polypeptides seen are: phosphoprotein (P); nucleocapsid (NC) and matrix protein (M). Solid line,  $^{32}\text{P}$  non-modulated; broken line,  $^{32}\text{P}$  modulated.

antibodies to measles virus overnight at  $4^\circ\text{C}$ . The antibody used for immune precipitation recognised all structural measles virus polypeptides found in the virion and in infected cells. The antigen-antibody complexes formed were then precipitated with *Staphylococcus aureus*, washed, dissolved in 1% SDS and 1% 2-mercaptoethanol and electrophoresed on SDS-PAGE in conditions designed to segregate measles virus polypeptides. In the conditions used, structural measles virus polypeptides were immunoprecipitated and co-migrated with purified measles virion polypeptides. Measles virus polypeptides were identified by tryptic peptide mapping<sup>11</sup>. Experimental controls showed that (1) measles virus antibody did not precipitate proteins in uninfected cells that migrated in positions of measles virus polypeptides and (2) sera free of antibodies to measles virus did not precipitate measles virus polypeptides found in infected cells.

Figure 1 shows the five structural polypeptides of measles virus demonstrated when HeLa cells were incubated only in fetal calf serum (NMOD) but not antibody. These are the haemagglutinin (HA), the phosphoprotein (P), the nucleocapsid (NC), the haemolysin (HL) and the matrix protein (M). Only the HA and HL were expressed on the cells' surfaces as detected by lactoperoxidase  $^{125}\text{I}$ -labelling of living cells<sup>12</sup> and previous observations<sup>13</sup>. In contrast, HeLa cells incubated with antibody to measles virus synthesised equivalent amounts of three of the five proteins, namely HA, NC and M as studied with  $^{35}\text{S}$ -methionine label (Fig. 1). Hence, in the experimental conditions used, modulation for 18 h or less, detectable amounts of HL, a surface protein, were markedly diminished by the presence of anti-measles antibody, whereas the other surface protein, HA, was unchanged. In addition, cytosol preparations from antibody modulated infected cells contained less P protein than unmodulated cultures. These results were reproducible and quantitated by both densitometer scans and counting the radioactivity contained within the labelled viral polypeptide. HL decreased by 70% or more and P by 80% or more in the preparations from modulated cells compared to those that were not modulated by antibody. Quantities of NC in modulated and unmodulated cells varied less than 10%. We performed a series of experiments in which the conditions of electrophoresis were altered (7–15% gels with and without urea), several human and rabbit sera containing measles antibody were used to modulate or precipitate, and purified IgG from these human and rabbit sera were used. All gave results similar to those shown in Fig. 1. Failure to decrease the amount of HA expressed on the surfaces of infected cells was unexpected, and probably due to the brief time (<18 h) for which infected cells were modulated. Previous experiments<sup>7</sup> showed that after 48 h of antibody-induced modulation, viral antigens were no longer detected on the cells' surface by either radiolabelled binding or immune cytotoxicity assays. When IgG from sera without antibody to measles virus was added to the cultures in identical conditions, neither HL or P protein were altered in concentration.

We next determined whether antibody(s) to non-viral determinants expressed on the surface of virus-infected cells also decreased the HL and/or P protein of measles virus. For these experiments, measles virus-infected HeLa cells were incubated with antibodies directed to the HeLa cell surface in conditions like those used for modulation. Although antibodies to HeLa cell surface components bound to antigens expressed on the membranes of virus-infected HeLa cells for up to 18 h in culture, the quantitative expression of the five viral polypeptides remained unaltered compared to cultures in which HeLa antibodies were absent or fetal calf serum was substituted. HL reacted correspondingly. Hence, the decreases in P and HL directly reflected the fact that antibodies to measles virus bound to measles virus determinants expressed on the cell surface.

P protein is a phosphorylated protein<sup>14</sup> and we next studied the incorporation of  $^{32}\text{P}$  into this protein during antibody modulation. Figure 2 shows a densitometer scan of immune precipitates obtained from cells labelled with  $^{32}\text{P}$  and autoradiographed. In unmodulated cells, P, NC and M polypeptides all incorporated  $^{32}\text{P}$ , indicating that these three measles virus structural proteins are phosphorylated. Comparatively, in modulated cells, only trace amounts of  $^{32}\text{P}$  were incorporated into P polypeptides while an enhancement of  $^{32}\text{P}$  was incorporated by the M protein. No difference in incorporation of  $^{32}\text{P}$  was noted for NCs studied in modulated and unmodulated cells. Labelling studies with  $^{32}\text{P}$  and  $^{35}\text{S}$  studies were run concurrently (Figs 1 and 2). Changes in the phosphorylation of M protein might alter NC recognition and alignment at the plasma membrane, thereby inhibiting viral maturation. Such alterations in phosphorylation could change the migrational characteristics of M protein on PAGE and may explain, in part, the differences in migration of M protein reported by others with SSPE material<sup>15,16</sup>.

Thus our results show that specific antibody can bind to the surface of an infected cell and not only strip off surface viral determinants but also alter viral polypeptide(s) found inside the cell not associated with the plasma membrane. It is not clear whether there is a decrease in synthesis, increase in degradation, or alteration of the P protein structure that would render it non-antigenic or change its mRNA. Nevertheless, it is clear that antibodies to a specific virus determinant on the cell surface can send a signal transmembrally that alters cytoplasmic viral events. Furthermore, the P protein, or its analogue in other viral systems, seems to be associated with the transcriptional complex<sup>17,18</sup>. The sequence of events described here could lead to alterations of measles viral synthesis leading to persistent infection.

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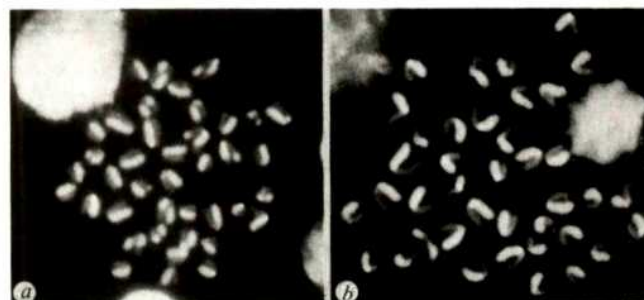
## ***In utero* sister chromatid exchange analysis for detection of transplacental mutagens**

FETAL exposure to agents which damage DNA can result in birth anomalies, cancer and inherited abnormalities. In the present report we describe a new approach for the detection of fetal DNA damage through the enumeration of sister chromatid exchanges (SCE) in mouse fetal chromosomes. SCE can be identified as reciprocal exchanges of fluorescent intensities between sister chromatids in metaphase cells which have divided twice in the presence of bromodeoxyuride (BrdU) (Fig. 1). Analysis of SCE has been shown to be a sensitive and reproducible means of detecting chemical mutagens and carcinogens<sup>1-9</sup>.

Differential chromatid staining of metaphase cells from maternal and fetal tissues was achieved when pregnant females were intravenously infused with BrdU for 24 h at gestational days 11, 15 and 19. SCE frequencies were simultaneously studied in fetal cells as well as in maternal bone marrow. Baseline SCE frequencies in fetal cells were generally lower than in maternal cells, ranging from 2.4 to 3.6 SCE per second replication cycle cell, and these values did not change significantly as a function of gestational age (Table 1, Fig. 1b).

To examine *in utero* SCE induction, three known mutagens (cyclophosphamide, CP; mitomycin C, MMC; adriamycin, ADM) were injected into pregnant females on day 13 of gestation (Table 1, Fig. 1a). Drug concentrations were selected which would induce similar levels of SCE for each agent in maternal cells (approximately 20 SCE per cell<sup>10</sup>). These same drug doses resulted in varying levels of SCE induction in fetal cells. Cyclophosphamide administration resulted in similar SCE induction in fetal and maternal cells; MMC resulted in fetal SCE levels which were slightly lower than those in maternal cells, while fetal SCE induction with ADM was only one third that seen in maternal cells (but significantly higher than baseline levels).

The doses of CP and MMC which resulted in a 5–10-fold increase in fetal SCE were well below the teratogenic doses for these drugs<sup>11,12</sup> while ADM, which showed the weakest induction of fetal SCE, is not teratogenic<sup>13</sup>. We therefore suggest that *in utero* SCE analysis should be used in addition to existing assays for screening fetal exposure to mutagens, carcinogens and teratogens which act at the level of DNA damage. *In utero* SCE analysis is relatively simple, rapid,



**Fig. 1** Photomicrographs of mouse fetal chromosomes. *b*, Second replication cycle cell showing baseline level of SCE; *a*, second replication cycle cell showing cyclophosphamide induced level of SCE. Two to four month-old female C57BL/6J mice (Jackson) were mated with male mice of the same age and strain. Gestational age was determined by counting the number of days after observation of vaginal plugs or by examination of fetuses at the time of killing. Pregnant mice were infused according to our previously described techniques at 50 mg per kg wt per h (refs 8, 15). After 23 h of intravenous infusion, mice were injected with 2.5 µg demecolcine (Colcemid, GIBCO) and killed 1 h later. Those mice treated with mutagens (CP, Mead, Johnson; MMC, Sigma; ADM, Adria Labs) were injected intravenously 1 h after the onset of the infusion. After killing, the entire uterus was excised and individual fetuses incubated in 0.2% collagenase (GIBCO). Vigorous pipetting in Eagle's minimal essential medium (MEM) resulted in single cell suspensions of fetal tissues. Cells were isolated from maternal bone marrow as described previously<sup>8,15</sup>. Cell suspensions were then swollen in 0.07 M KCl, fixed (methanol/acetic acid, 3:1) and dropped on to glass slides. Chromosome preparations were stained with 4'-6-diamidino-2-phenylindole (10 µg ml<sup>-1</sup>, (ref. 16)) and chromosomal analyses were performed with a Zeiss photomicroscope equipped with epi-illumination.

reproducible, requires small numbers of animals, allows simultaneous examination of the effect of agents on fetal and maternal cells, and may be more sensitive than previously described techniques such as the micronucleus assay<sup>14</sup> or measurement of chromosomal aberrations<sup>1</sup>.

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**Table 1** Baseline and mutagen-induced SCE frequencies in maternal and fetal cells

Gestational day*	Drug† (mg per kg)	Bone marrow‡	Embryos§
11	0	4.0 ± 0.4	2.7 ± 0.2 (4)
15	0	3.2 ± 0.4	2.4 ± 0.3 (4)
19	0	4.8 ± 0.5	3.6 ± 0.6 (3)
13	CP 10	23.4 ± 1.1	27.0 ± 1.4 (4)
14–15	CP 10	22.0 ± 1.4	25.0 ± 1.2 (4)
13	MMC 1	18.2 ± 0.9	14.2 ± 1.1 (3)
13	MMC 1	21.2 ± 1.0	15.8 ± 1.0 (3)
13	ADM 5	17.8 ± 1.2	6.3 ± 0.4 (4)
13	ADM 5	17.6 ± 0.9	6.5 ± 0.4 (4)

\* In cases where a range of days are indicated, gestational age was estimated by examination of fetuses at the time of killing.

† Drugs were intravenously injected in a volume of 0.1 ml.

‡ A minimum of 25 second replication cycle bone marrow cells were analysed for each mother.

§ A minimum of 10 second replication cycle cells were analysed for each fetus. Values in brackets indicate the number of fetuses analysed.

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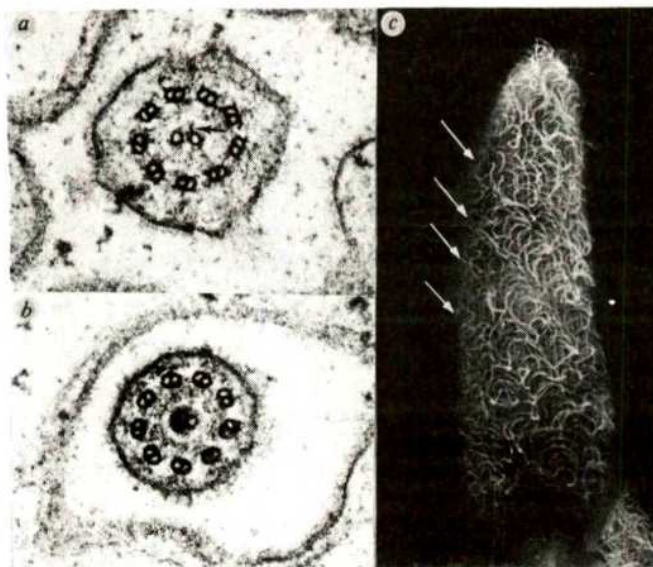


## The pair of central tubules rotates during ciliary beat in *Paramecium*

UNLIKE the one-dimensional movement of striated muscle, the beat of a cilium is typically three dimensional<sup>1</sup>. Thus, the dynein arms, which are situated on the peripheral tubules around the circumference as well as longitudinally along the cilium, must be temporally coordinated in their actions. The mechanism for coordination is not known. The present study was undertaken to see whether the pair of central microtubules exhibits any systematic movement during the ciliary beat. We conclude that the central pair of tubules rotates anticlockwise 360° per beat cycle and that this rotation may regulate the dynein arms. *Paramecium* cilia were used because: markers distinguish the two central tubules so that their orientation can be unambiguously determined; and the metachronal waves of cilia can be 'instantaneously fixed' for the analysis of sequential phases of the beat.

The force required for motion of cilia, eukaryotic flagella and sperm tails is generated by the dynein arms, which cause sliding of adjacent peripheral microtubule doublets<sup>2-4</sup>. The dynein arms have been shown to contain  $Mg^{2+}$ -ATPases<sup>5</sup>. The force generated by the arms can only cause the adjacent doublets to move tipward<sup>6</sup>. As the dynein arms are arranged in a ninefold radial symmetry, a force generated on one side will be cancelled by that on the opposite side. Thus, the physical arrangement necessitates a regulatory mechanism that allows some of the dynein arms to work while others do not. The central pair of the 9+2 microtubular structure is a candidate for at least part of this regulatory mechanism for several reasons. First, orientation of the central pair is correlated with beat direction. The central pair is orientated at right angles to the power stroke in a wide variety of metazoan cilia<sup>7,8</sup>. Gibbons<sup>8</sup> noted that in the Protozoa *Paramecium* and *Opalina*, the central-pair orientation was not uniform. Tamm and Horridge<sup>9</sup> then showed that the orientation of the central pair was perpendicular to the direction of bending, even when the effective stroke direction was changed. Second, various *Chlamydomonas* mutants missing one or both of the central tubules or even some accessory components of the central-tubule complex are found to be paralysed<sup>10</sup>. In these mutants, the dynein function, as assayed by peripheral microtubule sliding, remains normal<sup>11</sup>. Third, studies have indicated some interactions between the radial spoke of the peripheral doublets and the projections from the central pair. It has been suggested that such interactions convert sliding to bending<sup>12</sup>. When the radial spokes are deleted by mutations, the cilia are also paralysed<sup>11,26</sup>.

As in *Tetrahymena*<sup>13</sup>, the two central tubules in *Paramecium* can be distinguished by a 20-nm spur on one tubule (Fig. 1a). The two central tubules also terminate at different levels near the ciliary base (Fig. 1b), as shown by Dute and Kung<sup>14</sup>. Using both of these morphological markers, we have examined the orientations of central pairs near the bases of cilia on serial sections of the *Paramecium* surface. We have examined 119 central pairs in which both the insertion into the axosome and the 20-nm spur could be scored. A vast majority of these, 108 central pairs, show that the tubule which is inserted into the axosome also bears the spur (Fig. 1a, b). Of the remaining 11 cases, eight are ambiguous because different tubules were scored to have the spur in different serial sections. Only three cases show that the tubule with the spur is not inserted into the axosome. This observation makes the recent speculation by Hausmann and Fischer-Defoy<sup>21</sup> that there is alternate sliding of central tubules unlikely, and also increases our confidence in tubule identification. Using the spur and/or the axosomal insertion as markers, a vector can be drawn from the tubule without a marker to the tubule with the marker(s). The angular changes of this vector can then be studied.



**Fig. 1** a, Cross-section of a *Paramecium* cilium showing the 20-nm spur (arrow),  $\times 44,850$ . b, The same cilium as in a in more proximal section showing the tubule with the spur entering the axosome. The axosome is seen as an electron dense material around the tubule in cross-section,  $\times 44,850$ . c, Scanning electron micrograph of an instantaneously fixed *Paramecium* showing metachronal waves (arrows),  $\times 552$ . Wild-type *Paramecium tetraurelia*, 51s, were grown in Cerophyl medium bacterised with *Enterobacter aerogenes*<sup>15</sup>. The cells were washed then fixed while swimming in 4 mM  $CaCl_2$ , 1 mM Tris-HCl pH 7.2. An excess of instantaneous fixation solution (S. L. Tamm, personal communication) with final concentration of 2%  $OsO_4$ , 2% glutaraldehyde, 0.05 M Na-K phosphate pH 7.2 was pipetted on to cells and left at room temperature for 10–15 min. The cells were washed, post-fixed in 0.5% uranyl acetate and dehydrated through ethanol series. For scanning electron microscopy the cells were critical-point dried in 20- $\mu$ m mesh Nitex bags, put on stubs, coated with carbon-platinum and viewed on a JSMU3 scanning electron microscope. For transmission electron microscopy, cells were embedded in Epon-araldite<sup>16</sup> or in Spurr's<sup>17</sup> and sectioned on a Reichert OmU3 ultramicrotome. The sections were stained either with 0.5% uranyl magnesium acetate then lead citrate<sup>18</sup> or 1% potassium permanganate<sup>19</sup> then lead citrate<sup>20</sup>. Serial sections were photographed on a Phillips 300 electron microscope then enlarged photographically before examination.

Each beat cycle of the *Paramecium* cilia has a planar power stroke and a three-dimensional recovery stroke<sup>22</sup>. The cilia of an actively swimming *Paramecium* do not all beat in synchrony. Adjacent cilia in certain directions are slightly out of phase so that metachronal waves (Fig. 1c) are propagated. These waves give us an opportunity to study ciliary beat. Cilia at the same phase of beat form a line of synchrony parallel to the direction of the power stroke. Perpendicular to this line of synchrony, and in the direction of wave propagation, cilia are in progressively earlier phases of the beat (Fig. 2). Cilia just before the power stroke are at the wave crests (position 1, Fig. 2). The wave troughs (position 2) are made by cilia at the beginning of their recovery stroke which comprises most of the wave (positions 3–5). In *Paramecia* that have been 'instantaneously fixed', the waves are well preserved with cilia frozen in various phases of their beat (Fig. 1c). Our strategy has been to reconstruct the events during the ciliary beat cycle by examining the ultrastructure and orientation of central pairs of cilia along the line of wave propagation.

To examine the possible angular changes of the vector of the central pair, micrographs of the 20 to 30 serial sections near a *Paramecium* surface were traced, with cross-sections of all cilia outlined. The tubules with the spur and/or axosomal insertion were marked and the vectors drawn. Each cilium was numbered on each of the serial sections and the numbering was maintained through the series. An arbitrary line constant for all sections was



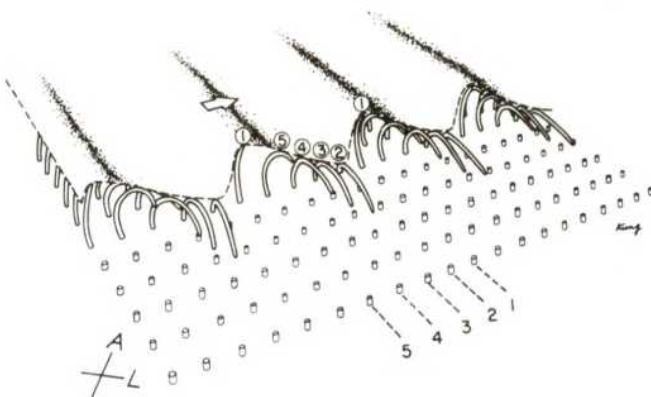
used to measure the angles of the vectors. The measured angles were grouped into nine  $40^\circ$  sectors. The classification of the angles into nine sectors is arbitrary but reflects the impracticability of measuring the angles with greater accuracy. The angle and coordinates for each cilium from all the serial sections were compiled into one map. The angles measured from all the cilia in the sections are not constant but span all the nine possible sectors. The best-fit set of parallel lines through groups of cilia with the same sector angle was drawn using a computerised co-variance analysis. In the more distal sections of the series, cilia could be seen in various cross, glancing or longitudinal views, depending on the phases of their beats. A survey of the pattern of these images of cilia enabled us to estimate the lines of synchrony. We found that the lines of synchrony estimated by this method parallel the lines of best-fit drawn from the angular data.

The orientation of each ciliary central pair near the base is plotted against its distance from a fixed computer-drawn line of synchrony (Fig. 3). Neighbouring cilia with central pairs in the same orientation are grouped (brackets in Fig. 3). In one typical case, shown in Fig. 3, the mean distance between neighbouring central pairs within groups is only  $1.4 \pm 0.7 \mu\text{m}$  (mean  $\pm$  s.d.; range  $0-3.5 \mu\text{m}$ ), while the mean distance between groups of the same orientation is  $6.7 \pm 1.2 \mu\text{m}$  (range  $6-12 \mu\text{m}$ ).

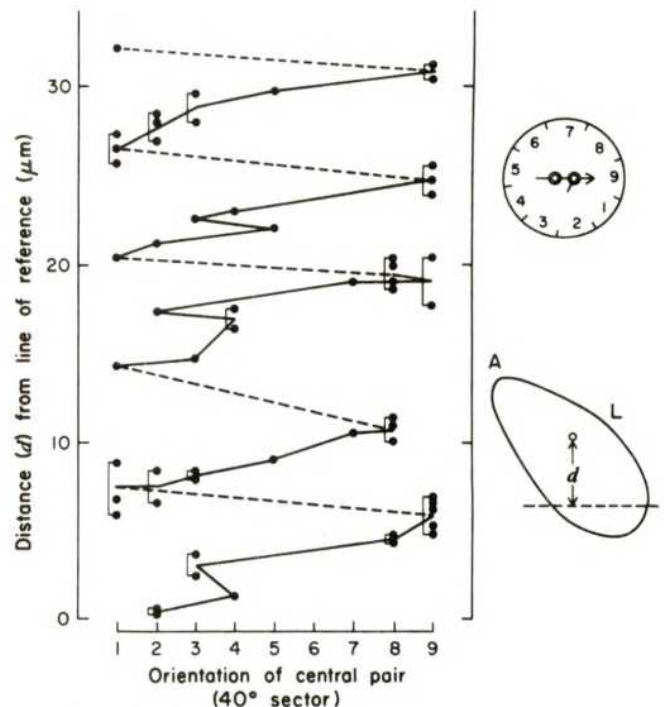
The central-pair orientation of cilia located along the perpendicular of the line of synchrony changes systematically in the anticlockwise direction (looking from tip to base) as one proceeds in the sequence of their beat cycle. This shift in orientation is continuous, with the exception of a few of the cilia in the cell in Fig. 3. The curvature of the *Paramecium* surface, the inaccuracy in aligning serial sections, the inability to score and include the vector for all the cilia and the imperfection of the metachronal waves themselves, all contribute to departure from the ideal.

We can also estimate the approximate wavelengths using the more distal sections described above, where cilia are seen in various views depending on the phases of their beat. In the case shown in Fig. 3, the wavelength is between 7 and  $12 \mu\text{m}$ . This is similar to the distance of  $6.7 \pm 1.2 \mu\text{m}$  (range  $6-12 \mu\text{m}$ ) between cilia having the same central-tubule orientation. Similar results have been obtained in four other *Paramecia*.

In the opposite direction from wave propagation, cilia are frozen at phases of the temporal sequence of their beat (Fig. 2)<sup>22</sup>. For a *Paramecium* fixed during forward swimming, this direction is from its anterior left to posterior right. Along this same direction we see that the central pairs rotate anticlockwise.



**Fig. 2** Stylised diagram of metachronal waves of cilia on the surface of *Paramecium*. Phases of ciliary beat are numbered 1-5. The power stroke is from 1 to 2 and the recovery stroke from 2, 3, 4, 5 back to 1. For a forward-swimming *Paramecium*, the power stroke is from anterior right to posterior left (refer to compass; A, anterior; L, left). This is also the direction of the lines of synchrony (dotted lines), along which cilia are at the same phase of their beat. Perpendicular to these lines of synchrony is the direction of wave propagation (broad arrow) which is opposite the direction of cilia at subsequent phases of their beat.



**Fig. 3** Orientations of the central pairs plotted against the distances of the cilia from a line of reference on the surface of *Paramecium* fixed during forward movement. The line of reference (dotted line of lower inset) parallels the lines of synchrony along which cilia are in the same phase of their beat (see text and Fig. 2). In forward swimming *Paramecium* this line is from its anterior (A) right to posterior left (L). The distances ( $d$ ) are measured in  $\mu\text{m}$ . The orientation of the central pair is represented by a vector drawn from the tubule without a spur to the one with (upper inset). The orientation is classified into nine categories as the vector falls into various  $40^\circ$  sectors. The sectors are numbered clockwise (looking from tip to base) and arranged such that the vector parallels the line of reference at sector 9. Neighbouring cilia with the same central-pair orientation within half a wavelength independently estimated from more distal sections (see text) are grouped (vertical brackets). The line is drawn through cilia or groups of cilia of successively increasing distance ( $d$ ) from the reference line. Note that, in general, the central-pair orientation is successively more clockwise as  $d$  increases. Five complete revolutions are seen. As  $d$  increases in the direction of wave propagation we encounter cilia frozen in successively earlier phases of the ciliary beat in this direction (see text and Fig. 2), and we conclude that the central pair rotates anticlockwise during the beat.

Thus, one simple explanation is that each central pair rotates anticlockwise during the beat. Furthermore, as the wavelength (estimated from distal sections) and the distance between groups of central tubules having the same orientation are similar, one may conclude that there is a  $360^\circ$  rotation with each beat cycle.

As shown in Fig. 3, there are far more central pairs orientated at sectors 8, 9, 1, 2 than at sectors 4, 5, 6, 7. Such an uneven distribution may be related to the temporal asymmetry of the ciliary beat cycle, that is, far more time is spent in the recovery stroke than in the effective stroke. We are now in the process of correlating the orientations of the central pairs to specific phases of the beat cycle.

An alternative interpretation of the data is that the central pair rotates in one direction for several sectors and then quickly rotates back, instead of continuously rotating in one direction. This would explain why few cilia orientate at certain sectors. However, the possibility is unlikely, as, without exception, the central-pair orientation of 27 individual cilia has been found to be progressively more anticlockwise when we examine the progressively more proximal sections of the same cilia. We have also examined  $0.25-0.5 \mu\text{m}$ -thick sections of *Paramecium* cilia under a high-voltage electron microscope and have found twists

in the central pairs. If the rotation of the central pair is generated at the proximal end, a twist may form and propagate distally along the cilium when the pair encounters some resistance along its length.

Regardless of whether the rotation is continuous or discontinuous, these results refer only to the proximal region of the central pairs. As we do not have markers on the peripheral tubules, we cannot determine their movement. Rotation of the whole axonemal assembly within which the central pair is fixed is not likely. Preliminary evidence from thick section high-voltage electron micrographs indicates that the assembly of the nine peripheral tubules also shows twists. However, the twist of the peripheral tubule assembly does not correlate with that of the central pair. Indeed, stereomicrographs of thick sections show that the twist of the central pair is opposite that of the peripheral tubule assembly in some portions of the cilia.

The 360° rotation of the central pair might be unique to a certain class of cilia or, on the other hand, it might be a phenomenon common to many types of cilia with the 9+2 structure. Variation in the orientation of the central pair has been reported. Satir<sup>23</sup> noted differences of more than 90° in the orientation of the central pair in active cilia from *Elliptio* gill fixed instantaneously. In *Opalina* Tamm and Horridge<sup>9</sup> reported that the orientation of the central pair is always at right angles to the direction of bending and that this orientation shifts by almost 90° at the trailing edge of the metachronal wave crests. As neither study used cilia with distinguishable central tubules, the angle variation observed is a minimal estimate.

We propose a model in which the central pair regulates the sliding of peripheral tubules. In this model, the rotation of the central pair acts as a distributor to signal peripheral tubule activity in sequence. This signal may be transmitted to the peripheral doublets through the radial spokes. The rotation of the central pair may generate the twist and cause its helical propagation towards the tip. This model would predict that the period of bend propagation is identical to the period of rotation. Observations with high-speed cinematography by Jarosch and Fuchs<sup>24</sup> of the flagellar motion in *Synura* are consistent with this prediction. This model may also provide the physical basis for the helical propagation of force-generating sites along the cilium postulated by Hiramoto and Baba<sup>25</sup>. The model can be tested by first showing a rotation of the central pair independent of peripheral tubule sliding. This may be done by using cilia whose dynein has been chemically inhibited or genetically removed. The model can be further tested by correlating the central-pair orientation with local dynein ATPase activation or cross-bridge formation.

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## Increased incidence of abnormal nasal cilia in patients with retinitis pigmentosa

RETINITIS PIGMENTOSA (RP) is the name given to a group of inherited eye disorders of unknown aetiology, whose symptoms are loss of night vision and constriction of the field of vision. The conditions are progressive and eventually in some cases blindness occurs<sup>1</sup>. Although there are certain syndromes which include RP, for example, the Laurence-Moon-Biedl<sup>1</sup>, or Cockayne's syndrome<sup>2</sup>, in most cases the disease is considered to be localised to the eye. However, a high incidence of deafness has always been associated with RP<sup>1,4</sup> and Massof and Finklestein (personal communication) have found that at least 15% of their cases have inner ear deafness not associated with presbycusis or acoustic trauma. As the outer limbs of photoreceptors are modified cilia<sup>3</sup> and the sensory epithelium of the inner ear is derived from ciliated epithelium, we wondered whether RP might be associated with a more general defect of ciliated structures. Accordingly we examined samples of nasal mucosa from 11 patients with RP and report here an increased incidence of cilia with abnormal axonemal microtubular structures, and also of compound cilia. Although our patient sample was small, it was heterogeneous and our findings may therefore apply to patients of different genetic type. If, as seems plausible, the ciliary abnormalities are related to the disease process in the eye, nasal mucosa would offer an accessible source of human material for the study of basic pathological processes in photoreceptors.

All our patients had been investigated at the Retinal, Genetic, and Electrodiagnostic Clinics of Moorfields Eye Hospital. Six of the cases had been called 'Usher's syndrome' (autosomal recessive RP with deafness<sup>4</sup>) although in some cases the deafness was not profound, or was of very early onset. In two cases, the

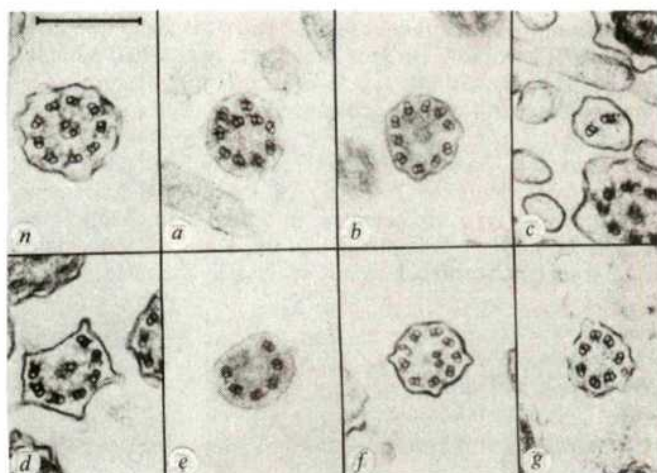
**Table 1** Relative numbers of abnormal axonemal patterns in patients and normal controls

Controls (N = 11)			RP patients (N = 11)		
9+2 & 9+0	'Tips'	Others (abnormal)	9+2 & 9+0	'Tips'	Others (abnormal)
254	6	13*	219	3	59*

The absence of the central two singlet microtubules (9+0) implies that the section was made near the base of the cilium, and is not an indication of abnormality. Likewise, sections through the tips of the cilia ('Tips', counted in separate column) show single microtubules symmetrically arranged about part or all of the circumference of the cilium. In the controls, between 0 and 19% of abnormalities were seen in various individuals: in the patients, the range was between 6 and 37%. See Table 2 for statistics.

\* $\chi^2 = 31.77$ , highly significant.





**Fig. 1** Transmission electron micrographs of normal and abnormal axonemes of cilia in transverse section. *n*, Normal 9+2 arrangement. *a-g*, Abnormal arrangements of microtubules, all from RP patients. Scale bar, 0.25  $\mu$ m.

inheritance was autosomal dominant: these patients did not complain of deafness and audiometry has not been carried out. In three patients, the RP seemed to be 'sporadic' and associated with deafness. In one of these deafness was inherited dominantly but the propositus was the only member of the family with RP. On this basis, and considering the nature of the disease in each patient, we classify the cases as eight recessive and three dominant. The 11 controls were reasonably well matched for sex and age. Samples of mucosa were taken from the inferior turbinate of the nose under either local anaesthesia (in RP patients and four of the controls) or general anaesthesia (the remainder of the controls). The tissue was immediately pinned flat on a piece of cork, with the ciliated surface uppermost, and placed in Karnovsky fixative to which 2 mmol of  $MgSO_4$  had been added to improve the visibility of the dynein arms<sup>5</sup>. After overnight fixation, the tissue was washed in buffer and postfixed in osmium tetroxide, followed by block staining with uranyl acetate. Tissue was dehydrated, mounted in 'Araldite' and sectioned. Sections were stained with uranyl acetate and lead citrate, and examined in a Phillips 201 electron microscope.

Electron micrographs of transverse sections of cilia were taken at a final magnification of 65,000–180,000. Only those cilia in which a microtubular pattern was clearly seen were counted. In some cases in which there were few precisely transverse sections of cilia, the gonioscopic stage was used to ensure that circular outlines were obtained.

In normal human material there are few departures from the classic 9+2 arrangement of the axonemal microtubules (Fig. 1, *n*). The abnormalities we saw in the patients' material consist of both increases (Fig. 1, *a* and *b*), decreases (Fig. 1, *c-g*) and irregular arrangements of the doublets (Fig. 1, *a* and *b*). There may also be displacement of the two central singlet microtubules (Fig. 1*f*). In one outline (not shown) we found five single microtubules centrally in the axoneme. Using the most conservative criteria, we find >20% of cilia from our patients to be abnormal, compared to 3% from the controls (Table 1).

**Table 2** Percentage of abnormal axonemal structures

Normal subjects	$\bar{X}$	s.e.m.
<i>N</i>		
11	3.55%	0.84%
(Subgroup with no nasal abnormality)		
5	3.21%	2.5%
RP patients		
11	22.84%	3.84%
(Subgroup with dominant inheritance)		
3	23.73%	4.8%

**Table 3** Compound cilia

	Control group	RP patients
No. of persons with no nasal abnormality	5	7
<i>a</i> Total length of section examined	607 $\mu$ m	734 $\mu$ m
<i>b</i> Length of ciliated cell		
mean	6.6	7.3
range	4.4–7.7	5.0–10.1
<i>c</i> Cilia/cell		
mean	70	89
range	36–125	23–113
<i>d</i> Estimated no. of cilia in sample	6,396	12,068
<i>e</i> No. of compound cilia in sample	0	39
<i>f</i> Proportion of compound cilia		
$\bar{X}$	0	0.86%
s.e.m.	—	0.32%
<i>g</i> Expected no. of compound cilia in control group	21 (from simple average), 55 (from value of $\bar{X}$ )	13–96 ( $\pm 2$ s.e.m.'s)

For each patient, several micrographs ( $\times 10,800$ ) of ciliated epithelium were examined, of length shown in *a*. The length of that single cell associated with the largest number of transverse sections of cilia *b* was then measured. We counted the number of these cilia *c*. From *a* and *b* we calculate the number of cells in the sample, and hence the number of cilia in the sample *d*. This method overestimates the number of cilia. The total number of compound cilia associated with the entire sample is given in *e*. For each patient we were able to determine the proportion of compound cilia, *f*, and thus obtain the s.e.m., which should be normally distributed. Hence we can make predictions *g* about the number of compound cilia we expect to see in the control group, on the assumption that the differences between the two columns are solely due to sampling. The predictions and the experimental result are so different that we can reject this hypothesis.

Both dominant and recessive cases showed the abnormality (Table 2). One patient with autosomal recessive disease had only 6% abnormal axonemes (and other abnormalities, see below). We do not yet know whether this is a sampling error, or whether recessive RP patients fall into more than one group. Though the mean values given in Table 2 show that normal subjects and patients fall into separate groups, we cannot tell from this preliminary survey what the population ranges may be.

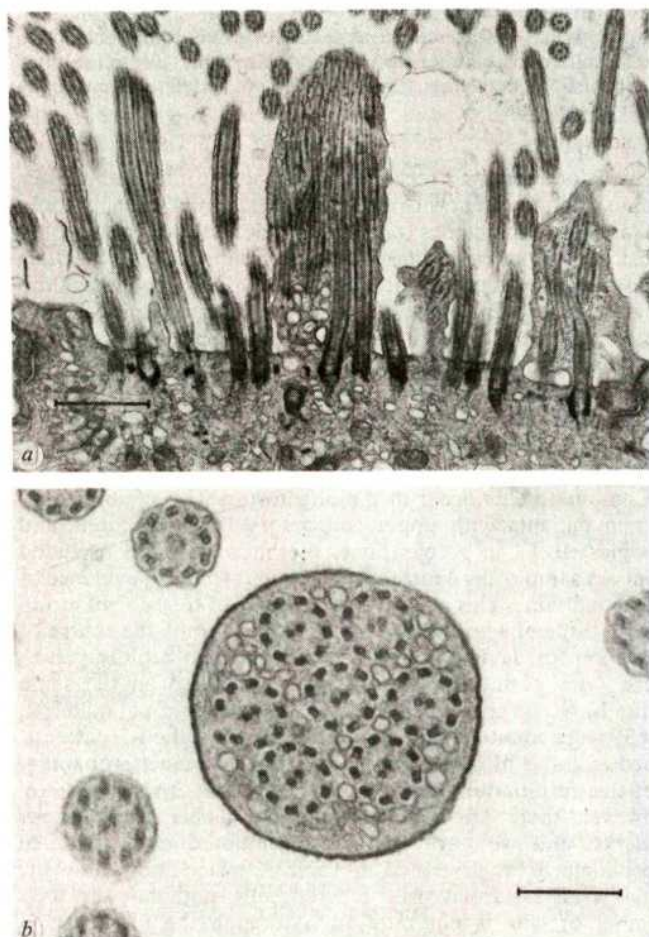
Large structures containing many sets of microtubules were seen in many of our patients. Although the incidence of such compound cilia was low ( $\sim 0.3\%$ ) the appearances were bizarre and striking (Fig. 2).

Compound cilia occur in the olfactory system of some fish<sup>6</sup> and in patients with upper respiratory tract infections and allergies<sup>7</sup> (B.F., in preparation). We have therefore excluded from our sample any control or RP patient who had evidence of such conditions. This reduced the number of the control group to five, three of whose tissue was obtained during the course of operation for deviated nasal septum, and two healthy volunteers. Four of the RP patients were excluded on the same grounds. No compound cilia were seen in the normal material, but 39 were counted in the sections made from the RP patients' mucosa, in five of the seven cases. The Fisher exact probability that this result could occur by chance is very low,  $P=0.026$ . However, there are very few compound cilia, even in our patients, and we have therefore attempted more detailed morphometry, as described in Table 3, which shows that the patients have normal sized ciliated cells, and that the total number of cilia is not reduced. Although the incidence of compound cilia is small, given the length of ciliated epithelium examined, there is only a very small probability that the patients and normal material could be drawn from the same population. In one of the two patients in whom we saw no compound cilia, a large sample was obtained, and, using the data from Table 3, we calculate that we should have seen 10 compound cilia, if the incidence was the same in this patient as in the remainder. It is



thus possible that RP patients do not form a uniform population so far as the incidence of compound cilia is concerned.

Apart from deafness, 10 of our cases of RP have difficulties with balance. In one further case (not reported here) we have found abnormalities of the cilia of the uterine tube. Moreover, in Cockayne's syndrome, there is an unexplained predisposition to respiratory infection which could be due to a ciliary defect<sup>2</sup>. The defects of cilia in our patients may be generalised and the loss of photoreceptors could be due to the same basic cause. However, there is evidence that the pigment epithelium, and not the rods, is defective in some animal retinal dystrophies<sup>8</sup> and perhaps in some patients<sup>9,10</sup>. In this connection it seems possible that RP patients do not form a homogeneous group, and one or other or both of the defects shown in Figs 1 and 2 may be absent. This would be important in efforts to subdivide the group of diseases called retinitis pigmentosa, and to assess the number and frequency of abnormal genes<sup>11,12</sup>. Our results do not show immediately how the abnormality detected could produce RP. The growth of rod disks, or the provision of metabolites for the rod outer segments may be associated with the disposition of microtubules in the narrow ciliary neck. The biochemistry of cilia and outer limbs is strikingly similar in many ways<sup>13-17</sup>; and thus cilia may form a useful model for the inaccessible photoreceptors. Research into RP has been handicapped by the lack of normal human ocular tissue, and the almost complete absence of suitable human pathological material<sup>18</sup>. In contrast, it should be possible to obtain large quantities of normal and abnormal human cilia fairly easily.



**Fig. 2** *a*, Transmission electron micrograph showing longitudinal sections through compound cilia, from a patient with dominantly inherited RP. Scale bar, 1  $\mu$ m. *b*, Transmission electron micrograph of compound cilium in transverse section. Note the 7 microtubular patterns with a 9+2 arrangement. The single axoneme at '11 o'clock' has an additional peripheral microtubule. (RP patient, autosomal recessive inheritance.) Scale bar, 0.25  $\mu$ m.

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*Note added in proof:* We have recently investigated three patients with X-linked disease. All have ~40% abnormal axonemes, but two are females, in whom retinal functional defects are mild<sup>11,12,20</sup>, implying that the degree of abnormality of photoreceptors and cilia is not necessarily the same.

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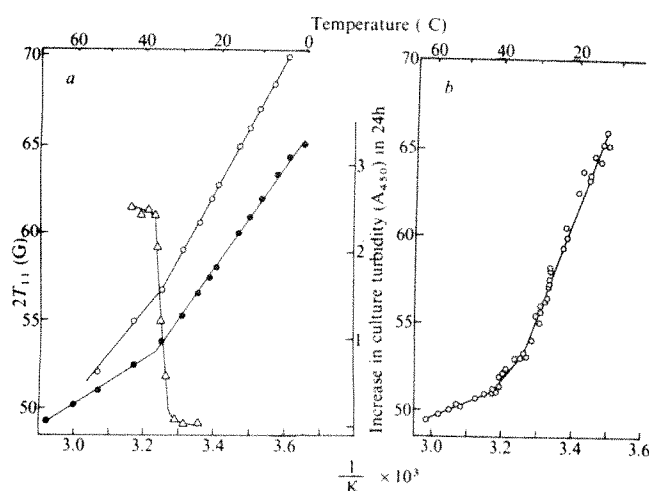
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## Membrane fluidity of a fatty acid auxotroph grown with palmitic acid

HOMEOVISCIOUS adaptation, whereby the fatty acid composition and unsaturation of complex lipid bilayers in membranes may be adjusted to maintain fluidity irrespective of growth temperature, has been demonstrated for *Escherichia coli*<sup>1</sup>, *Acholeplasma laidlawii*<sup>2</sup> and *Bacillus stearothermophilus*<sup>3</sup>. Even at high temperatures, a minimum amount of unsaturated fatty acid is required for growth of an *E. coli* auxotroph<sup>4</sup>, and generally, for *A. laidlawii* B, at least half the lipids must be in the fluid or disordered state to allow normal growth<sup>5</sup>. Unlike the fatty acid auxotrophs of *E. coli* and *A. laidlawii* used in previous investigations of membrane fluidity<sup>2,4,5</sup>, a fatty acid auxotrophic *Butyrivibrio* sp. (strain S2) isolated from the ovine rumen was found to be unable to synthesise long-chain fatty acids and incorporated palmitic acid, without desaturation, into lipids of the plasmalogen type partially as a new long-chain dicarboxylic acid<sup>6</sup>. This gave an opportunity to study the lipid composition of a microorganism with a defined fatty acid availability, and also to study the state of fluidity of its membranes in the complete absence of acyl chain unsaturation. We report here a study of membrane lipid fluidity in *Butyrivibrio* S2 using the electron spin resonance (ESR) probe 5-doxylstearic acid. Considerable rigidity of hydrocarbon chains is demonstrated but a phase change or structural reorganisation occurs at the lowest temperature, allowing optimal growth of the organism.

After incorporation of [1-<sup>14</sup>C]palmitic acid into *Butyrivibrio* S2 the long-chain <sup>14</sup>C-labelled hydrophobic moieties produced by methanolysis of the complex bacterial lipids (Table 1) consisted of: (1) about 7% cetyl alcohol; (2) some 40% of the dimethyl





**Fig. 1** *a*, Temperature dependence of maximum hyperfine splitting ( $2T_{11}$ ) for 5-doxyzystearic acid incorporated into whole cells (○) or extracted lipids (●) of *Butyrivibrio* S2 and the relationship between growth temperature and the increase in culture turbidity ( $\Delta A_{450}$ ) in 24 h. *b*, Temperature dependence of maximum hyperfine splitting ( $2T_{11}$ ) for 5-doxyzystearic acid incorporated into multilamellar structures of dipalmitoyllecithin. The gel straight line is a least squares fit to the experimental data. The discontinuities at 33.5 and 41 °C are consistent with the pre- and main transition temperatures reported previously<sup>14</sup>. 5-Doxyzystearic acid was mixed with the extracted bacterial lipids or dipalmitoyllecithin in chloroform/methanol (2:1 v/v) in the molar ratio of ~1:300. The mixed lipid was readily dispersed in D<sub>2</sub>O by a method described previously<sup>15</sup>. In spin-label experiments using whole cells, residual cysteine from the growth medium which could destroy the nitroxide radical<sup>3,16</sup>, was removed by washing the cells four times with 0.1 M phosphate buffer, pH 7.4. The 5-doxyzystearic acid (0.5–1% of the calculated dry weight of lipid in the cells, assuming an average molecular weight of 1,500) was dried down from chloroform/methanol (2:1 v/v) solution in a round-bottom flask and the dry film was incubated with the cell suspension with shaking for 15 min. ESR measurements were carried out with a Varian E 104A (X band) spectrometer equipped with a variable temperature accessory ( $\pm 1^\circ$  heating rate  $20^\circ\text{C h}^{-1}$ ). Samples were contained in 0.1 ml glass or quartz capillaries accommodated within standard 4 mm quartz ESR tubes. Bacterial growth was determined turbidimetrically ( $A_{450}$ ).

ester of a new type of long-chain dicarboxylic acid which had been formed in the bacterium by a dehydrogenation condensation reaction involving two palmitic acid molecules, and has recently been identified as 15,16-dimethyltriacontan-1,30-dioic acid (diabolic acid); (3) 41–42% of long-chain aldehyde (as the dimethylacetal derived from plasmalogen), consisting predominantly of palmitaldehyde but with a little stearaldehyde; and (4) a smaller long-chain fatty acid methyl ester component (9–11%) consisting largely of palmitic acid but with a trace of stearic acid. The complex lipids also contained appreciable quantities of esterified but unlabelled butyric acid.

As with only palmitic acid available for growth, no unsaturation or cyclopropane moieties occur in the hydrophobic chains of *Butyrivibrio* S2, the bacterium possesses no fatty acid desaturase system. Furthermore, as the organism cannot synthesise long-chain fatty acid<sup>6</sup>, it is likely that the low concentrations of stearic acid and stearaldehyde present are derived from trace impurities of stearic acid in the palmitic acid or other medium components. No radioactivity was detected in the stearic acid when the organism was grown in the presence of [ $1-^{14}\text{C}$ ]palmitic acid, indicating that it had not been formed by chain elongation.

The fluidity of the organism's membranes and the lipids extracted from them was examined by ESR using the probe 5-doxyzystearic acid (*N*-oxyl-4',4'-dimethylloxazolidine derivative of 5-ketostearic acid, I(12, 3); Synvar). At all temperatures examined the spectra of the spin label present in either whole cells or the lipid dispersion were typical for highly anisotropic

motion. Table 2 shows a comparison of the published values of the maximum hyperfine splitting  $2T_{11}$  for 5-doxyzystearic acid bound to various cell membranes at temperatures between 15 and 25 °C; it is clear that *Butyrivibrio* S2 grown with a palmitic acid supplement shows a degree of order in its membrane matched only by the rigid, specialised purple membrane of *Halobacterium* spp. At the growth temperature of the organism (39 °C) the maximum hyperfine splitting was  $2T_{11} = 55.5$  G (Fig. 1a), a value similar to that observed with the same label in dipalmitoyllecithin bilayers below the  $T_c$  (in the gel phase) (Fig. 1b), in which the hydrocarbon chains are packed in an orderly crystalline lattice.

The temperature dependence of the maximum hyperfine splitting ( $2T_{11}$ ) of the spectrum of 5-doxyzystearic acid bound to whole cells was compared with that obtained when the same label was incorporated into the lipids extracted from such cells. Plots of  $2T_{11}$  against the reciprocal of absolute temperature (Fig. 1a) showed discontinuities at 34.5 °C for the whole cells and 35.5 °C for the extracted lipids, indicating some type of phase change or structural reorganisation in membrane lipids. This temperature coincided with the lowest temperature at which optimal growth occurred in a palmitate-supplemented medium (Fig. 1a). The single break in the Arrhenius plot (Fig. 1a) probably reflects the relative simplicity of the lipid hydrocarbon chains, which exhibit little variation in chain length, and no unsaturation or cyclopropane substitution as is usually seen in bacterial lipids. Clearly this latter heterogeneity in the fatty acid chains is unnecessary for bacterial growth, a conclusion also recently reached by Silvius and McElhaney<sup>8</sup>, using *A. laidlawii* B grown in the presence of antilipogenic agents.

It is significant that the break in the Arrhenius plot (Fig. 1a) coincides with the minimum temperature that allows optimal growth in a palmitate-supplemented medium. What are the structural changes that occur in the lipid bilayer at that temperature and are apparently necessary for membrane viability and vigorous growth of *Butyrivibrio* S2? The membrane lipids of *Butyrivibrio* S2 possess no unsaturation except the vinyl ether unsaturation in the plasmalogen chains. However, there is little evidence that plasmalogen when substituted for acyl chains can increase the fluidity of lipid bilayers<sup>9,10</sup>.

**Table 1** Long-chain hydrophobic moieties present in *Butyrivibrio* S2 grown with a palmitic acid supplement

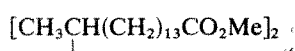
Hydrophobic moieties produced	Structure	% Present	
		Expt 1	Expt 2
Alcohol	C16:0	6.77	6.00
Diabolic acid			
dimethyl ester	(C16:0) <sub>2</sub>	41.03	37.99
Dimethylacetal	C16:0	40.15	41.46
	C18:0	2.65	2.15
Fatty acid			
methyl ester	C16:0	8.91	11.06
	C18:0	0.48	1.33

*Butyrivibrio* S2, an obligate anaerobe, was cultured in a virtually fatty acid-free liquid medium containing 0.4% (w/v) galactose, as described previously<sup>6</sup> (medium 3). The palmitic acid ( $30\ \mu\text{g ml}^{-1}$ , and in some experiments [ $1-^{14}\text{C}$ ]labelled) required to promote growth was dispersed in this medium with the aid of sodium taurocholate ( $300\ \mu\text{g ml}^{-1}$ ) except where extracted lipids or washed cells were to be used for ESR experiments, when a growth-limiting concentration of fatty acid ( $20\ \mu\text{g ml}^{-1}$ ) was dispersed in the medium by ultrasonication. Cultures were inoculated with 5% (by vol) inoculum, grown to maximum turbidity in the same medium, and were incubated at 39 °C for 16–18 h. Bacterial cells were collected by centrifugation ( $20,000g$ ; 20 min) and lipids were extracted and analysed<sup>6</sup>. The complex lipids were hydrolysed with 5% (w/v) HCl in methanol (90 °C; 2 h) which converted any esterified long-chain carboxylic acids into their methyl esters and long-chain aldehydes (plasmalogens) into dimethylacetals while any alcohols were liberated as such. Quantification of the hydrophobic moieties was carried out by radioactivity measurements after TLC<sup>6</sup> and the chain lengths present were determined by GLC.

**Table 2** Maximum hyperfine splitting  $2T_{11}$  of 5-doxylstearic acid spin label bound to various cell membranes

Membrane system	Temperature (°C)	$2T_{11}$ (G)	Reference
Human erythrocytes	23	55–60	17, 18
Human lymphocytes	23	53.7	17
L cells	23	54.7	17
Sarcoplasmic reticulum vesicles	22	53.8	19
<i>E. coli</i> membrane vesicles	20	56.7	20
Brush border membrane vesicles	23	60	Hauser, H., unpublished
Beef heart mitochondria	23	55.6	
<i>Mycoplasma laidlawii</i> membranes	15	58.0 (cells grown at 15 °C)	21
membranes	15	62.5 (cells grown at 37 °C)	
<i>Halobacterium halobium</i> (purple membrane)	15	63.7	
	20	63.0	22
	25	62.0	
<i>Butyrivibrio</i> S2	15	65.0	Present work
	20	63.0	
	25	61.0	

There are two possible ways in which some limited degree of hydrocarbon chain motion could occur in the lipid bilayer. First, it has been shown here and previously<sup>11</sup> that many of the complex glycolipids and phospholipids produced by members of the genus *Butyrivibrio* contain at least one esterified butyryl group per molecule. Such short fatty acid chains are likely to increase the fluidity of a lipid bilayer. Second, the long-chain dicarboxylic acid (diabolic acid) occurring as an integral esterified part of the membrane's phospholipid contains two vicinal methyl groups substituted in the centre of the methylene chain<sup>7</sup>. Such methyl groups are again likely to perturb the orderly packing of the hydrocarbon chain in much the same way that anteiso methyl branched fatty acid molecules pack less readily and consequently have a much lower capillary melting point than their straight-chain or isobranched homologues<sup>12,13</sup>. Indeed, the melting point of 41 °C observed<sup>7</sup> for the dimethyl ester of the diabolic acid



isolated from palmitic acid grown *Butyrivibrio* S2 cells is much less than that predicted from the melting points of the homologous series of straight-chain dicarboxylic acid diesters. To a certain extent the role of the diabolic acid could be equivalent to that believed to be played by the high levels of anteiso branched fatty acyl groups found in psychrophilic microorganisms when compared with their closely related mesophilic counterparts<sup>12</sup>. These acids, together with the greater percentage of unsaturated fatty acyl chains, help to maintain membrane fluidity at the lower growth temperature of the psychrophilic organisms. However, this fluidising effect of the vicinal methyl groups in diabolic acid might be partly counterbalanced by the very long hydrocarbon chain which may span the membrane bilayer thus reducing the cooperative lipid motion.

We believe that the present naturally-occurring auxotroph has survived in the rumen through its ability to make use of the abundance of saturated fatty acid (both palmitic and stearic acid) and *trans* octadecenoic acid formed by biohydrogenation occurring in this environment. When grown on palmitic acid, the bacterium incorporates this fatty acid exclusively into the lipid part of its membranes, resulting in a very rigid membrane

structure as indicated by the large hyperfine splitting (Table 2). Although *Butyrivibrio* S2 has no capacity to shorten chains or desaturate the fatty acids available from the environment or convert these into a cyclopropane form, it may nevertheless decrease the rigidity of its lipid bilayers by: (1) introducing butyryl groups produced by the fermentation of hexoses, and (2) introducing anteiso-type branching by a novel type of condensation reaction between two palmitic acid chains. These structural features are probably responsible for some kind of molecular motion in the lipid bilayer, which, though rather restricted, is sufficient to maintain the functions of the membrane at growth temperatures above 34 °C. The ESR measurements indicate that the membranes of the organism have a tightly packed, highly ordered lipid bilayer even at the physiological growth temperature of 39 °C. This suggests that in *Butyrivibrio* S2 a more limited degree of hydrocarbon chain motion is required for membrane viability than has been found in other bacteria.

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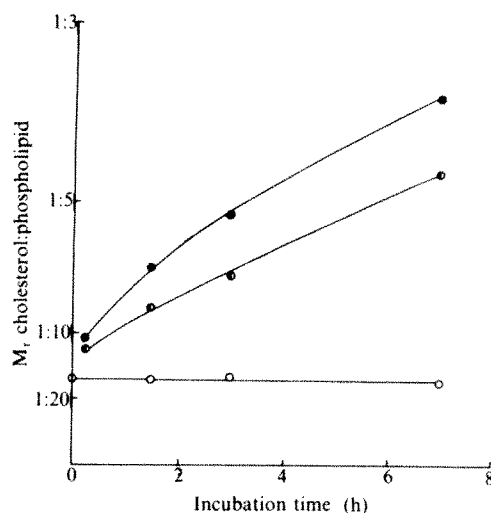
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## Cholesterol modulates activity of calcium-dependent ATPase of the sarcoplasmic reticulum

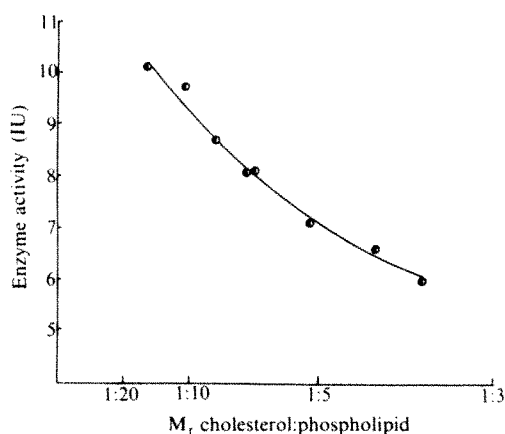
BIOMEMBRANES consist of an asymmetric lipid bilayer matrix into which and around which the various proteins are situated. The proteins may be attached to the outside of the lipid bilayer (extrinsic proteins), but in many cases the proteins (intrinsic proteins) are embedded within, and can span, the bilayer. Associated with this is the idea that in many cases the lipid matrix is in a fluid condition in which the lipids are essentially above their transition temperature ( $T_c$ ) and able to diffuse along the bilayer length. The perturbation introduced into the lipid bilayer by the presence of an intrinsic protein has recently been discussed<sup>2,3</sup>. Some workers<sup>4,5</sup> have suggested that intrinsic proteins, for example the  $\text{Ca}^{2+}$ -ATPase of the sarcoplasmic reticulum, carry with them, even when excess bulk fluid lipid occurs, a



**Fig. 1** Change in the ratio of cholesterol to phospholipid in SR membranes during incubation in the presence of cholesterol-rich liposomes. SR vesicles ( $1.5 \text{ mg protein ml}^{-1}$ ) were incubated in the absence of liposomes (○), with  $1 \text{ mg dipalmitoyllecithin-cholesterol per ml}$  (○●), or  $2 \text{ mg dipalmitoyllecithin-cholesterol per ml}$  (●) in a medium consisting of  $0.1 \text{ M KCl}$ ,  $5 \text{ mM histidine buffer pH } 7.4$ ,  $100 \text{ U ml}^{-1}$  penicillin-G and  $100 \mu\text{g ml}^{-1}$  streptomycin sulphate. The incubations were performed in siliconised flasks gassed with nitrogen and shaken gently in a water bath maintained at  $20^\circ\text{C}$ .

shell of immobilised lipid, referred to as an annulus, which controls the enzyme activity. The shell is said to exclude cholesterol so that cholesterol molecules do not influence the enzyme activity. We report here the use of cholesterol-enriched liposomes to reversibly vary the content of cholesterol in the sarcoplasmic membranes. We show in contrast to the previous work that as the cholesterol content of the membrane varies so does the activity of the  $\text{Ca}^{2+}$ -ATPase.

Sarcoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase is an example of an intrinsic protein where a single lipid shell of some 30 lipid molecules is bound relatively tightly to the protein. This immobilised layer or annulus is assumed to co-exist with and to be separate from the bulk lipid. The intrinsic protein is then envisaged to exist as a protein-lipid complex undergoing rotational and lateral diffusion within the remaining fluid lipid bilayer<sup>4</sup>. This has led to the suggestion that a similar situation occurs with a major class of other membrane proteins<sup>6</sup>. Methods of changing the cholesterol content of biomembrane systems *in vitro* have now been developed. Incubation of cholesterol-rich liposomes with a variety of cells and subcellular organelles has been shown to cause a rapid exchange of the sterol and there is a



**Fig. 2**  $\text{Ca}^{2+}$ -dependent ATPase activity of SR as a function of the ratio of phospholipid to cholesterol in the membrane. Enzyme assays were performed as described in the text.

net transfer of cholesterol on creation of a concentration gradient<sup>7,8</sup>. We have exploited this system to alter the cholesterol content of sarcoplasmic reticulum (SR) by incubating membrane vesicles with cholesterol-rich liposomes. The effect of introducing cholesterol on the activity of  $\text{Ca}^{2+}$ -dependent ATPase in the natural biomembrane system was examined.

SR isolated from rabbit hind leg muscle has a relatively simple protein and lipid composition<sup>9,10</sup>. More than 60% of the total membrane protein is  $\text{Ca}^{2+}$ -dependent ATPase which is responsible for pumping  $\text{Ca}^{2+}$  from the sarcoplasm into the cisternae of the SR during the recovery phase leading to muscle relaxation. Calcium uptake by SR vesicles has been shown to be stoichiometrically coupled to ATP hydrolysis<sup>11,12</sup>. The membrane-bound enzyme has a molecular weight of about 110,000 and it is partly interpolated into the lipid bilayer<sup>13</sup>.

Preliminary experiments investigated the rate and extent of cholesterol incorporation into SR vesicles. SR, prepared by the method of Martonosi *et al.*<sup>14</sup> and further purified by sucrose density gradient centrifugation<sup>15</sup>, was incubated with cholesterol-rich liposomes. These were prepared by co-lyophilising dipalmitoyllecithin and cholesterol (molar ratio ( $M_1$ ), 1:2) from benzene:methanol 95:5 (v/v) and then sonicating the mixture in a sealed vessel under nitrogen for 12 min at  $30^\circ\text{C}$  in distilled water. After sonication, the liposome suspensions were centrifuged at  $50,000g$  for 60 min to remove undispersed lipid and titanium dislodged from the sonicator probe. Aliquots of the incubation mixture were removed at appropriate time intervals and cholesterol-rich liposomes were separated from SR vesicles by the following procedure. The suspension was diluted with 2 vol.  $0.1 \text{ M KCl}$ ,  $5 \text{ mM histidine buffer pH } 7.4$ , and centrifuged for 30 min at  $50,000g$ . The membrane fraction sedimenting as a pellet was resuspended in 20% (w/v) sucrose buffered to pH 7.4 with  $5 \text{ mM histidine}$  and layered at the interface between 27% sucrose,  $5 \text{ mM histidine}$ , pH 7.4 and  $0.1 \text{ M KCl}$ ,  $5 \text{ mM histidine}$ , pH 7.4. Centrifugation of the gradient for 2 h at  $100,000g$  separates any remaining cholesterol-rich liposomes. These band at the interface of  $0.1 \text{ M KCl}$  and 20% sucrose layers and the SR is recovered as a pellet at the bottom of the gradient.

Figure 1 shows changes in the  $M_1$  of cholesterol:phospholipid in the purified membrane fractions during incubation with cholesterol-rich liposomes. Cholesterol was extracted from the SR by the method of Rose and Oklander<sup>16</sup> after saponification of the lipid, and assayed by quantitative gas chromatography using an internal standard of cholestane. The amount of cholesterol transferred to the membranes can be seen to vary both with the concentration of cholesterol-rich liposomes and with incubation time. No loss of phospholipid<sup>17</sup> or protein<sup>18</sup> from the SR could be detected and there was no incorporation of dipalmitoyllecithin (Table 1) into the membrane as judged from the pattern of fatty acid methyl esters separated by gas chromatography<sup>19</sup>. In separate control experiments, membrane vesicles were incubated with pure dipalmitoyllecithin liposomes which, in contrast to cholesterol-rich liposomes, could not be separated from the SR by the method described. Fusion of the cholesterol-rich liposomes with the membrane is excluded on the basis of freeze-fracture electron microscopy which shows two distinct populations of vesicles, one with prominent intramembrane particles, believed to represent intrinsic protein of the SR, and the other devoid of particles, presumably the phospholipid liposomes. Adhesion of liposomes to the vesicles did not effect  $\text{Ca}^{2+}$ -ATPase throughout the 7-h incubation.

The relationship between  $\text{Ca}^{2+}$ -dependent ATPase activity and the cholesterol content of the SR membrane is shown in Fig. 2. Calcium-dependent ATPase activities were measured at  $32^\circ\text{C}$  in the presence of an ATP regenerating enzyme system. The assay medium consisted of  $0.1 \text{ M triethanolamine buffer pH } 7.4$ ,  $0.1 \text{ M KCl}$ ,  $5 \text{ mM MgCl}_2$ ,  $0.5 \text{ mM EGTA}$ ,  $3 \text{ mM Mg-ATP}$ ,  $1.25 \text{ mM phosphoenolpyruvate}$ ,  $0.2 \text{ mM NADH}$ , pyruvate kinase (20 IU) and lactate dehydrogenase (20 IU) in a total volume of 3 ml. The SR was preincubated in the assay medium for at least 10 min and the  $\text{Ca}^{2+}$ -independent ATPase activity determined:  $\text{Ca}^{2+}$ -dependent ATPase was then measured

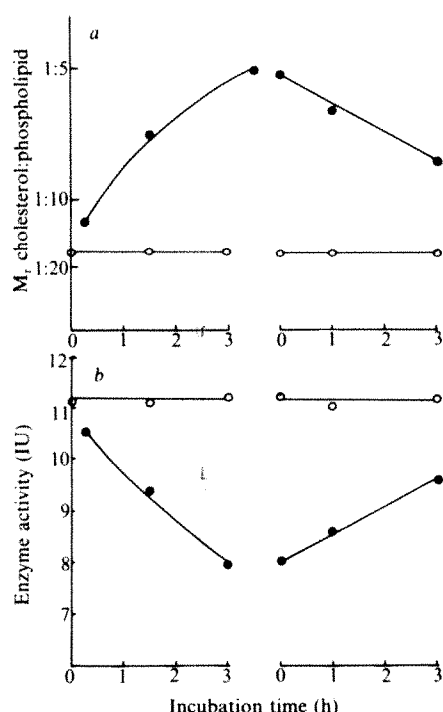
**Table 1** Analysis of SR vesicles before and after incubation

Sample	Concentrations of CRL in incubation medium (mg ml <sup>-1</sup> )	Incubation time (h)	Protein concentration (mg ml <sup>-1</sup> )		Phospholipid mg ml <sup>-1</sup> )		Protein/phospholipid ratio		
SR Vesicles, control	0	0	1.51±0.02		1.07±0.02		1.41±0.02		
	0	7	1.48±0.03		1.05±0.02		1.41±0.03		
SR cholesterol supplemented	1	0	1.49±0.02		1.05±0.01		1.42±0.02		
	1	7	1.48±0.03		1.06±0.02		1.40±0.03		
SR cholesterol supplemented	2	0	1.50±0.02		1.07±0.02		1.40±0.02		
	2	7	1.48±0.02		1.05±0.02		1.41±0.02		
Analysis of major fatty acids (% total)									
			16: alde	16:0	18:0	18:1	18:2	18:3	20:4
SR control	0	7	4.0±0.3	27.7±0.5	12.8±0.3	19.5±0.5	24.5±0.6	3.1±0.3	8.4±0.5
SR cholesterol supplemented	2	7	4.1±0.3	27.6±0.6	13.1±0.4	19.5±0.4	24.8±0.6	2.8±0.3	8.4±0.5

Values given are mean ± s.e.m. for four samples. CRL, cholesterol-rich liposomes.

following addition of CaCl<sub>2</sub> to give a final assay concentration of 0.55 mM. The rate of oxidation of NADH was monitored on a recording spectrophotometer.

It can be seen that Ca<sup>2+</sup>-stimulated enzyme activity is decreased in direct proportion to the cholesterol content of the membrane over the range examined. Cholesterol is known to restrict the motion of disordered fatty acyl chains of pure phospholipid dispersions or phospholipids in biological membranes<sup>20,21</sup>. This results in a reduction in enthalpy of the lipid thermotropic phase transition and a decrease in fluidity of membranes at temperatures above the phase transition temperature. Recent physical studies of SR membranes using high angle X-ray diffraction, NMR spectroscopy and calorimetric techniques have suggested that the lipids are all in a liquid-crystalline state<sup>22,23</sup> above 10 °C. The effect of cholesterol on Ca<sup>2+</sup>-dependent ATPase is therefore consistent with an effect on the fluidity of the SR membrane.



**Fig. 3** Restoration of Ca<sup>2+</sup>-ATPase activity on removal of added cholesterol. SR (1.5 mg protein per ml) was incubated in the presence of 2 mg dipalmitoyllecithin-cholesterol per ml for 3 h followed by incubation with 1 mg of egg-yolk lecithin per ml for 3 h (●), or in the absence of liposomes (○). *a*, Cholesterol content of the membranes at intervals during incubation; *b*, corresponding Ca<sup>2+</sup>-ATPase activities.

To investigate this further and to discount the possibility that cholesterol interacts directly with the enzyme to cause an irreversible loss of activity, cholesterol was removed from cholesterol-supplemented membranes. Figure 3 shows results for a membrane preparation incubated with cholesterol-rich liposomes and subsequently with liposomes of egg-yolk lecithin to partially re-extract the incorporated cholesterol.

Qualitatively similar results were obtained when SR was incubated with lower liposome concentrations (1 mg ml<sup>-1</sup>). Removal of cholesterol from the SR membranes results in a corresponding recovery of Ca<sup>2+</sup>-dependent ATPase activity. This confirms that cholesterol does not irreversibly inactivate the enzyme and that the presence of cholesterol in the membrane causes a modulation of the Ca<sup>2+</sup>-ATPase activity. The only interpretation possible for these results is that if an annulus as envisaged by Warren *et al.*<sup>5</sup> does exist around this protein then cholesterol is not excluded. This is in contrast to the conclusion of these workers.

The existence of such an annulus has been recently questioned. Studies have shown that almost full enzymatic activity can be recovered after replacing virtually all of the membrane phospholipids with suitable detergents<sup>24</sup>. Furthermore, biochemical<sup>25</sup> and biophysical evidence (for example <sup>2</sup>H NMR and ESR spectroscopy)<sup>26</sup> is considered to be inconsistent with the assumptions inherent in the annulus concept.

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## ATP induces nucleotide permeability in rat mast cells

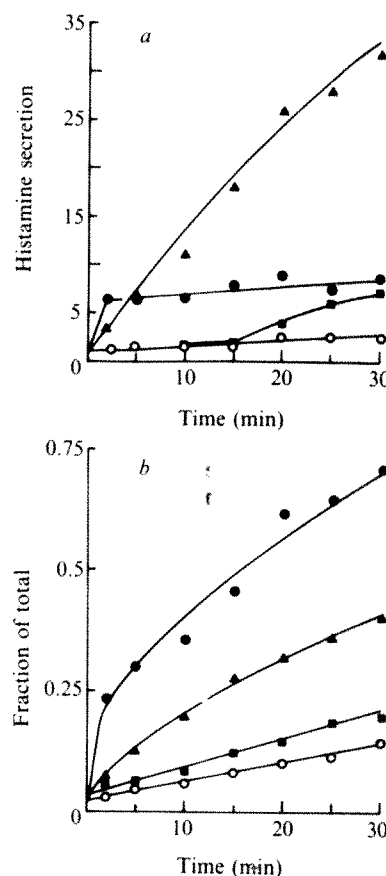
ATP is one of several agonists which stimulate  $\text{Ca}^{2+}$ -dependent exocytotic degranulation of mast cells, causing the release of histamine and other mediators of immediate hypersensitivity<sup>1-3</sup>. The concentration-effect relationship of ATP on mast cells is characterised by the sharp onset of self-inhibition as the concentration is raised above the optimum for histamine secretion<sup>4</sup>. We have previously shown that stimulation of mast cells with IgE-directed ligands (antigen, concanavalin A), chymotrypsin and compound 48/80 is accompanied by an increased turnover of phosphatidylinositol<sup>5</sup> similar to that observed for  $\text{Ca}^{2+}$ -mediated responses in other tissues<sup>6</sup>. At concentrations of ATP which cause inhibition we have observed a reduction in phosphatidylinositol turnover to less than control levels (unpublished results). We show here that the inhibition by ATP is correlated with an extensive leakage of phosphorylated metabolites from the cells.

The effective agonist form of ATP is  $\text{ATP}^{4-}$  (refs 3, 4); that is, the ATP which remains unbound to the divalent cations  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$ , which are normal components of physiological salt solutions. Optimal concentration for  $\text{ATP}^{4-}$ -mediated secretion is about  $3 \mu\text{M}$ . With  $\text{ATP}^{4-}$  applied at  $5 \mu\text{M}$ , inhibition of secretion due to itself<sup>4</sup> or other ligands is almost total<sup>7</sup> and a parallel inhibition of glycolytic metabolism has also been observed<sup>8</sup>. Rat peritoneal mast cells were preincubated with  $^{32}\text{P}$ -phosphate, washed free of external radioactivity and purified to better than 95% homogeneity (see Fig. 1 legend). When we applied ATP to these cells at a concentration optimal for secretion (Fig. 1a) (at which  $\text{ATP}^{4-}$  was calculated to be  $2.9 \mu\text{M}$ ), we found that there was an increased efflux of  $^{32}\text{P}$ -labelled material from the cells (Fig. 1b). As the concentration of free ATP was raised to  $8.6 \mu\text{M}$ , histamine secretion was prevented (Fig. 1a) but the loss of  $^{32}\text{P}$ -labelled material increased (Fig. 1b). ATP applied to the cells at concentrations below the optimum for secretion had only a small effect on the loss of label (Fig. 1a, b).

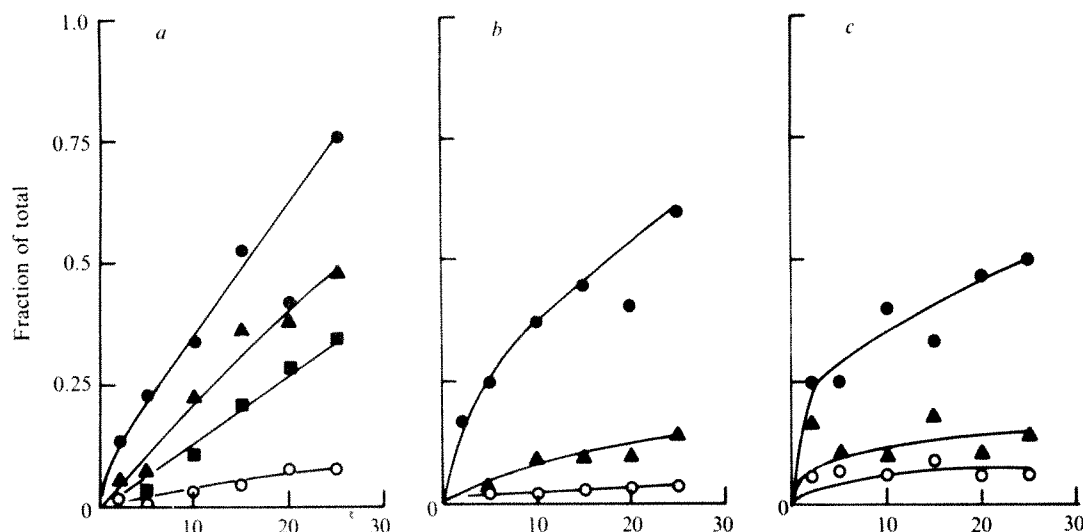
We separated the extracellular material into three fractions by passage through activated carbon (to separate nucleotides)<sup>9</sup> and by extraction into isopropylacetate, after formation of the phosphomolybdate complex to separate inorganic phosphate from organic phosphates (probably phosphorylated intermediates of glycolytic metabolism)<sup>10</sup>. Figure 2 shows the appearance of radioactivity in these three fractions as functions of ATP concentration and time.

At low concentrations ( $1.4 \mu\text{M}$  free ATP, sub-optimal for secretion) the labelled material appearing outside the cells is entirely in the form of inorganic phosphate. As the concentration of free ATP is raised through  $2.9 \mu\text{M}$  (optimal for secretion) to  $8.6 \mu\text{M}$  (inhibitory) the rate of loss of inorganic phosphate from the cells increases, but in these conditions a considerable loss of nucleotides and other  $^{32}\text{P}$ -labelled material also occurs. This loss of metabolites is not a consequence of exocytotic degranulation, as extensive histamine secretion due to application of compound 48/80 is not accompanied by the appearance of labelled metabolites in the extracellular medium.

Loss of metabolites on this scale would be quite sufficient to account for the inhibitory effects of externally applied ATP on glycolysis<sup>8</sup> and the ATP-induced phosphatidylinositol response. Exogenous ATP is known to permit the leakage of intracellular anions (including nucleotides) from some transformed (but not



**Fig. 1** a, Time course of histamine secretion from purified rat peritoneal mast cells, induced by various concentrations of ATP. b, Time course of ATP-induced loss of  $^{32}\text{P}$ -labelled metabolites from mast cells. Rat peritoneal cells were obtained by peritoneal lavage and suspended in 2 ml of a balanced salt solution (composition previously described<sup>5</sup>). The mixed cells were loaded with  $^{32}\text{P}$ - $\text{PO}_4$  ( $100 \mu\text{Ci ml}^{-1}$ , carrier-free) by incubation at  $37^\circ\text{C}$  for 2 h. The mast cells were then isolated from the extracellular  $^{32}\text{P}$ - $\text{PO}_4$  and purified to >95% homogeneity by centrifugation ( $400g$ , 15 min) through a cushion of Ficoll (30%) as described elsewhere<sup>5</sup>. The purified mast cells were then washed twice by centrifugation and suspended in the balanced salt solution at  $5 \times 10^5$  cells per ml. The cells were incubated at  $37^\circ\text{C}$  with concentrations of ATP as indicated. At various times  $250 \mu\text{l}$  were removed and quenched into 1 ml of ice-cold saline. After centrifugation at  $4^\circ\text{C}$  ( $400g$ , 5 min) samples of supernatant were removed for measurement of radioactivity and of histamine (method as previously described<sup>17</sup>). Both the total and free ATP concentrations are indicated; free ATP concentrations were calculated from equilibrium considerations with  $K_{(\text{Mg}, \text{ATP})} = 10^{4.28}$  and  $K_{(\text{Ca}, \text{ATP})} = 10^{3.94}$  (ref. 4). ATP concentration ( $\mu\text{M}$ ) (total/free):  $\circ$ , 0/0;  $\blacksquare$ , 50/1.4;  $\blacktriangle$ , 100/2.9;  $\bullet$ , 300/8.6.



**Fig. 2** Time course of ATP-induced loss of inorganic phosphate (a), non-nucleotide organic phosphates (such as sugar phosphates) (b), and nucleotides (c), from prelabeled mast cells. After incubation for various times in the presence of ATP, the supernatants were collected as described in Fig. 1, and samples fractionated as described in the text. Free ATP concentration ( $\mu\text{M}$ ):  $\bullet$ , 8.6;  $\blacktriangle$ , 2.9;  $\blacksquare$ , 1.4;  $\circ$ , 0.

untransformed) cell lines<sup>11</sup>, but we stress that this effect of ATP occurs at concentrations more than two orders of magnitude above that which causes leakage of metabolites from mast cells. The effect on mast cells shows specificity for ATP; 2-deoxy ATP showed a small effect when applied at 500  $\mu\text{M}$  (total concentration), and CTP, GTP, ADP, adenosine and the synthetic ATP analogue, adenylylimidodiphosphate, were without effect when applied at 1 mM (total); they did not prevent the action of ATP.

The affinity of the ATP receptor on mast cells is of the order of  $10^6 \text{ l mol}^{-1}$  but this might be modulated by the presence of divalent cations. In the absence of divalent cations nucleotide loss from mast cells began with  $\text{ATP}^{4-}$  applied at 1  $\mu\text{M}$  and was maximal with  $\text{ATP}^{4-}$  at 3  $\mu\text{M}$ , conditions which permit only the loss of inorganic phosphate in the presence of  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  (see Fig. 2). The divalent cations which were present in our experiments to act as buffers for the ATP and to permit secretion may thus additionally confer protection on the cells against the detrimental effects of ATP. We cannot say whether the ATP receptor responsible for enhanced anion permeability is the same as that which stimulates phosphatidylinositol turnover,  $\text{Ca}^{2+}$  and  $\text{Na}^{+}$  fluxes<sup>12</sup> and histamine secretion. The enhanced permeability does not extend to macromolecules (leakage of lactate dehydrogenase was less than 5%) and is in no sense lytic.

Perhaps the most interesting observation is the graded response to molecules of increasing molecular weight as the concentration of ATP applied to the cells is increased. Thus, free ATP at 1.4  $\mu\text{M}$  enhances the permeability of the mast cells only to inorganic phosphate, which increases as the concentration of free ATP is raised to 2.9  $\mu\text{M}$ . It is only above this level that sugar phosphates and nucleotides begin to appear. This indicates that as the concentration of external ATP is raised, a change occurs in the filtration properties of the anion channels through which the intracellular metabolites emerge. One possibility is the successive involvement of channels of different specificity; alternatively, it would result from variation in the dimensions of a single channel type. A possible mechanism would involve channel formation by the aggregation of transmembrane monomeric units in the manner which has been discussed with respect to the channels formed by the polyene antibiotics<sup>13</sup> and alamethicin<sup>14</sup>. In the latter case it has been argued that enlargement of pre-formed channels occurs by the recruitment of additional monomers into the oligomeric conducting unit. The conductivity of the junctional membrane channels of *Chironomus* salivary gland varies inversely with the concentration of intracellular  $\text{Ca}^{2+}$  (ref. 15) and this correlates with variation in the size exclusion limits for transfer of a series of fluorescent dyes between neighbouring cells<sup>16</sup>. In this case, too, it has been suggested that the variable filtration characteristics could arise from alterations in effective channel size.

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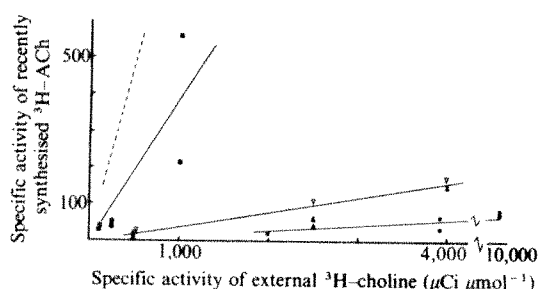
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## Choline transport is not coupled to acetylcholine synthesis

It has been proposed<sup>1–3</sup> that choline transport, particularly that performed by the so-called high-affinity system, is obligatorily coupled to acetylcholine synthesis and therefore only choline that has just entered by the transport mechanism is acetylated. If this were so it would imply that the enzyme choline acetyltransferase is bound close to the transport sites on the membrane. The problem is important because of its implications for the molecular organisation of the synapse and also because subcellular fractionation studies<sup>4,5</sup> show fairly clearly that, at the ionic strengths prevailing inside the cell, choline acetyltransferase is soluble and not bound to the plasma or synaptic vesicle membrane (as would be expected if the hypothesis of obligatory coupling is true). The evidence for this is indirect and relies on the finding that the half-maximal concentration for acetylation of choline and its analogues *in situ* approximates more nearly to the half-maximal concentration of their transport than of their acetylation by the isolated enzyme<sup>1</sup>. However, this



**Fig. 1** Utilisation of external choline for ACh synthesis. Synaptosomes were preincubated as described in the text with  $8 \mu\text{M}$   $^{14}\text{C}$ -glucose ( $304 \mu\text{Ci } \mu\text{mol}^{-1}$ ) and then washed and incubated for 10 min with different concentrations ( $\bullet$ ,  $0.2 \mu\text{M}$ ;  $\blacktriangle$ ,  $0.8 \mu\text{M}$ ;  $\blacksquare$ ,  $10 \mu\text{M}$ ) of choline at various specific activities. Dashed line shows theoretical 100% curve. Each point is the result of separate incubation.  $\nabla$ , Incubation carried out in  $24 \text{ mM K}^+$  which depolarises and causes ACh release. Ordinate, specific activity of the  $^3\text{H}$ -ACh synthesised during the 10 min incubation with  $^3\text{H}$ -choline.  $^{14}\text{C}$ -ACh present in synaptosomes just before the addition of the pulse was  $533 \pm 56 \text{ d.p.m. per mg}$  (s.e.m.,  $n = 10$ ) and rose to  $917 \pm 28 \text{ d.p.m. per mg}$  (s.e.m.,  $n = 20$ ) during the 10-min choline pulse.

need only imply a coupling between transport and synthesis if the latter is rate controlling, and from a survey<sup>6</sup> of the rates of release and synthesis of acetylcholine (ACh) it seems that the activity of choline acetyltransferase is in considerable excess of normal requirements. Certain exceptions to the obligatory coupling of transport and synthesis have been noted but these involve synthetic analogues of choline<sup>7</sup> or unusual preparations<sup>8,9</sup>. Reduction of the acetyl-CoA levels reduces ACh concentrations without a concomitant reduction in choline transport<sup>10</sup> but neither this nor the previous studies exclude the possibility that the residual ACh synthesis remains coupled; that is, obligatorily dependent on choline that has just been transported across the membrane. We describe here an experiment which we believe demonstrates that choline that has been recently transported has no privileged access to choline acetyltransferase in the synaptic terminal. Instead it mixes with the pre-existing pool of choline and therefore there is no coupling between transport and synthesis.

The principle of the experiment is that if there was obligatory coupling only choline originally outside the synaptic terminal would be acetylated. If, therefore, the terminals are exposed to pulses of choline of different specific  $^3\text{H}$ -radioactivities, the specific activity of the choline moiety of ACh synthesised inside the terminal during the pulse should be exactly that of the choline to which the terminals were exposed. Quantitatively, a plot of the specific activity of the recently synthesised ACh

against the specific activity of the choline pulse will have a slope numerically equivalent to its contribution towards ACh synthesis. This will be unity if coupling is obligatory, and will decrease as the contribution of the transported choline to the total amount of choline utilised decreases.

To distinguish recently synthesised ACh from pre-existing stores and estimate its specific radioactivity, acetyl CoA was labelled by pre-incubation of synaptosomes with  $^{14}\text{C}$ -glucose. The specific  $^{14}\text{C}$  radioactivity of the ACh synthesised during the  $^3\text{H}$ -choline pulse will be identical to the specific  $^{14}\text{C}$  radioactivity of the acetyl CoA during the period of the pulse. As specific activities are ratios of radioactivity to moles the denominators cancel and the specific  $^3\text{H}$  radioactivity of the recently synthesised ACh is given by the ratio of  $^3\text{H}$ - to  $^{14}\text{C}$ -labelled ACh synthesised during the pulse, multiplied by the specific  $^{14}\text{C}$  radioactivity of the acetyl CoA.

Nerve terminals from cerebral cortex (synaptosomes) were used because although they are heterogeneous with respect to the transmitter, their metabolic characteristics are understood better than alternative preparations of cholinergic synaptosomes. The characteristics of carrier-mediated choline transport in these particles compare favourably with that in intact tissues<sup>11</sup>. The synaptosomes were isolated by conventional procedures, introduced into and washed twice with a physiological saline containing  $180 \text{ mM NaCl}$ ,  $3 \text{ mM KCl}$ ,  $2 \text{ mM CaCl}_2$ ,  $2 \text{ mM MgCl}_2$  and  $8 \text{ mM NaPi}$  ( $\text{pH } 7.2$ ) but without glucose.  $^{14}\text{C}$ -glucose ( $8.2 \mu\text{M}$ ,  $304 \mu\text{Ci } \mu\text{mol}^{-1}$ ) was then added and the synaptosomes incubated at  $37^\circ\text{C}$  for 60 min to label the acetyl CoA. The low concentration of glucose was used to achieve a satisfactory incorporation of radioactivity into acetyl CoA with reasonable economy of isotope. Some experiments were carried out at a glucose concentration of  $0.5 \text{ mM}$  with similar results. These and a comparison of the uptake of  $^3\text{H}$ -choline and conversion to  $^3\text{H}$ -ACh at higher glucose concentrations are given in Table 1.

After the incubation an aliquot of the suspension was taken to determine the initial value of  $^{14}\text{C}$ -ACh. The  $^3\text{H}$ -choline pulse was then added so that the final concentrations of choline were  $0.2$ ,  $0.8$  and  $10 \mu\text{M}$  and at each concentration there were three different specific activities in the range  $50$ – $10,000 \mu\text{Ci } \mu\text{mol}^{-1}$ . After 10 min the synaptosomes were sedimented by a rapid ( $5\text{-s}$ ) centrifugation in a Janetski centrifuge and the washed pellets taken for analysis.  $^3\text{H}$  and  $^{14}\text{C}$  radioactivities in ACh were determined after extraction and thin layer chromatography<sup>12</sup>. The specific radioactivity of the  $^{14}\text{C}$ -acetyl CoA was measured after trichloroacetic acid extraction by removing the choline with two washes of sodium tetraphenylboron ( $10 \text{ mg ml}^{-1}$ ) in vinyl acetonitrile and then allowing the acetyl CoA to acetylate enzymatically radioactive  $^3\text{H}$ -choline of known specific activity using the choline acetylase of a brain acetone powder. The

**Table 1** Utilisation of external choline for acetylcholine synthesis in various conditions

Choline concentration ( $\mu\text{M}$ )	Glucose concentration (mM)	Influx of $^3\text{H}$ -choline in 10 min (pmol per mg)	Synthesis of $^3\text{H}$ -ACh in 10 min (pmol per mg)	Fractional utilisation of external choline $\pm$ s.e.m. ( $n$ )	Influx of $^3\text{H}$ -choline in 10 min expressed as a fraction of total endogenous choline
0.2	0.008		2.0	0.013	
0.2	0.5	7.6	2.0	$0.031 \pm 0.06$ (4)	0.02
0.2	10.0	8.0	4.9		
0.5	0.5		5.5	$0.039 \pm 0.001$ (4)	
0.5 (depolarising medium)	0.5			0.048	
0.5	10.0	16.0	9.5		0.05
0.8	0.008			0.029	
0.8 (depolarising medium)	0.008			0.038	
10	0.008		36	0.360	
10	0.5	79	50	$0.485 \pm 0.029$ (4)	0.2
10	10.0	90	51		

The influx of  $^3\text{H}$ -choline and synthesis of  $^3\text{H}$ -ACh were measured at the various choline and glucose concentrations in separate experiments<sup>14</sup> and are expressed as pmol per mg synaptosomal protein. The fractional utilisation of external choline was calculated from the slopes of plots made as in Fig. 1. In the last column the influx of choline in 10 min is expressed as fraction of the total synaptosomal content of endogenous choline determined in separate experiments<sup>14</sup> as  $400 \text{ pmol per mg synaptosomal protein}$ .

specific  $^{14}\text{C}$  radioactivity of the synaptosomal acetyl CoA was  $2.2 \mu\text{Ci } \mu\text{mol}^{-1}$  and it did not change significantly during the pulse.

The results for a typical experiment are shown in Fig. 1. As expected the points fall on straight lines passing through the origin. Duplicate data show reasonable agreement. Incubating the synaptosomes in a depolarising medium<sup>13</sup> so that they released ACh had little effect. The slopes are much less than unity at choline concentrations of  $0.2\text{--}0.8 \mu\text{M}$ , but increase to about 0.5 at choline concentrations of  $10 \mu\text{M}$ . This means that for the synthesis of ACh there is a substantial dilution of the external choline by the choline pre-existing in the terminal, and therefore there does not seem to be any coupling of transport to synthesis. Furthermore as the choline concentration was increased out of the 'high-affinity' range the slopes increased, indicating a greater utilisation of external choline. This is the opposite to what would be expected if the high-affinity uptake alone was coupled to ACh synthesis, as at higher choline concentrations its effect would be swamped by the low-affinity uptake. In fact the utilisation of external choline agrees tolerably well with the contribution of influx to the total synaptosomal choline pool, as can be seen from the summary of results in column 6 of Table 1.

Synaptosomes derived from cerebral cortex are not all cholinergic but this will not affect our results as non-cholinergic synaptosomes do not synthesise ACh and will therefore not have contributed to the data. We have considered the possibility that efflux of choline from the synaptosomes was diluting the  $^3\text{H}$ -choline in the medium. Choline efflux was measured and although some dilution occurred the qualitative aspects of the experiment were preserved. The uptake of  $^3\text{H}$ -choline and its conversion to  $^3\text{H}$ -ACh are slightly but not markedly reduced (Table 1, columns 3 and 4) by the low concentrations of glucose used in this study. It could be argued that, even though these are relatively unaffected by low glucose concentrations, there might be interference with other aspects of synaptosomal functioning that indirectly affect the results. However, the overall pattern of the coupling is maintained at a glucose concentration differing by 20-fold (that is from  $8 \mu\text{M}$  to  $0.5 \text{ mM}$ ) so it seems reasonable to suppose that this pattern will be preserved at the 2–10-fold higher concentration of glucose conventionally used.

Although these experiments were not designed to demonstrate the existence or otherwise of high-affinity transport systems specifically associated with cholinergic synaptosomes<sup>15–18</sup> they do not support this notion. If there was such a system it would follow that the proportional utilisation of external choline at low concentrations would exceed the utilisation at high concentrations. The reverse is the case; moreover the contribution of transport (measured over the whole population) to the total synaptosomal choline pool is in reasonable agreement with its contribution to ACh synthesis in cholinergic synaptosomes. High-affinity uptake, if it exists, makes only a minor contribution to the synthesis of the transmitter.

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## Effects of catecholamines, ATP and ionophore A23187 on potassium and calcium movements in isolated hepatocytes

IN addition to their well known effects on hepatic gluconeogenesis and glycogenolysis, adrenaline and noradrenaline cause a transient net loss of potassium from the liver of many species<sup>1</sup>. Studies with guinea pig and rabbit liver slices have shown this to be a consequence of a predominantly  $\alpha$ -adrenoceptor-mediated increase in the potassium permeability ( $P_K$ ) of the hepatocyte membrane<sup>2–4</sup>. It has recently become possible to isolate hepatocytes in high yield and with cation levels and metabolic capabilities superior to those of slices, and approaching that of the intact tissue<sup>5</sup>. We have now confirmed that the effects of catecholamines on potassium movement can be shown with such cells, and we have obtained evidence to support the suggestion that the potassium loss which follows  $\alpha$ -adrenoceptor activation in guinea pig liver cells is a consequence of an increase in potassium permeability triggered by a rise in intracellular calcium. The mechanism can be blocked by quinine, as in human erythrocytes<sup>19</sup> and barnacle photoreceptors<sup>20</sup>.

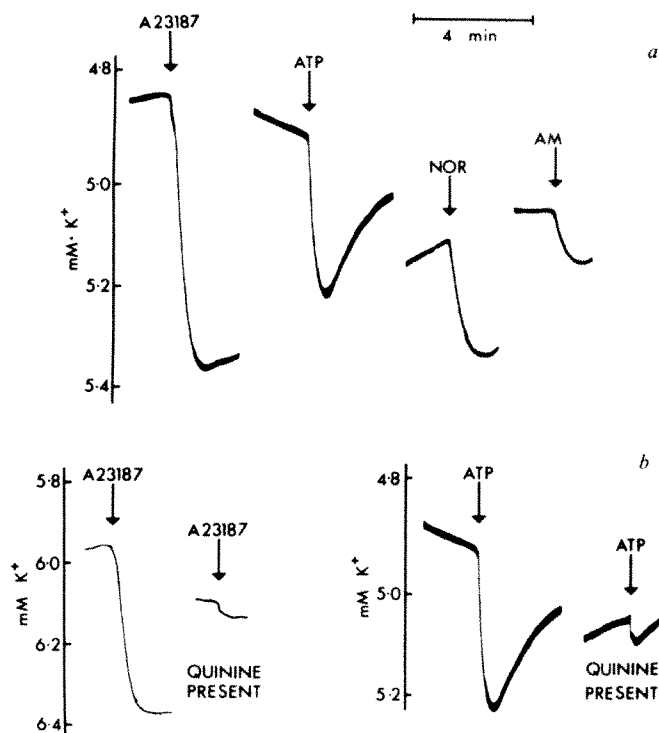
Haylett<sup>4</sup> first raised the possibility that the increase in  $P_K$  in liver cells is a consequence of a rise in the concentration of calcium ions in the cytosol (see also refs 6, 7). Evidence for the two processes envisaged in this proposal, an effect of  $\alpha$ -receptors on intracellular calcium, and control of  $P_K$  by cytosolic calcium, has been obtained in various tissues including salivary and lacrimal glands<sup>6–12</sup>. On such a scheme we could expect the divalent cation ionophore A23187 to initiate potassium loss by increasing cytosolic calcium, and hence  $P_K$ . This was tested with hepatocytes isolated by Seglen's method<sup>13,14</sup> from the liver of male Hartley guinea-pigs (220–300 g). The cells were equilibrated at  $37^\circ\text{C}$  for 30–60 min in Eagle's medium<sup>15</sup> (Wellcome) containing (mM): NaCl, 116; KCl, 5.4;  $\text{CaCl}_2$ , 1.8;  $\text{MgSO}_4$ , 0.81;  $\text{NaH}_2\text{PO}_4$ , 0.96;  $\text{NaHCO}_3$ , 25, and (mg  $\text{l}^{-1}$ ): amino acids, 805; vitamins, 8.1; glucose, 1,000; L-glutamine, 292; phenol red, 10. This was supplemented with 2% albumin (fraction V, Sigma). The pH was maintained at 7.4 by circulating 5%  $\text{CO}_2$  in  $\text{O}_2$  above the cell suspension which was shaken at 120 strokes per min. In some experiments (including those with  $^{45}\text{Ca}$ ), samples of the suspension were filtered to eliminate the largest clumps of cells and then centrifuged at 50g for 2 min. The cell pellet was resuspended either in normal Eagle's medium or in a low-Ca, low-Mg variant (Ca, 0.1 mM; Mg, 0.05 mM) sometimes containing  $^{45}\text{Ca}$  ( $0.2 \mu\text{Ci ml}^{-1}$ ) but without albumin. The viability of the cells was checked before and after each experiment by their ability to exclude Trypan blue (4%). By this test between 80 and 85% of the cells were viable at the time when drugs were applied.

Figure 1 shows that A23187 caused a rapid loss of potassium from the isolated cells, as did the  $\alpha$ -agonists amidephrine<sup>16,17</sup>, phenylephrine (not illustrated) and noradrenaline. ATP was also found to be effective. This is in keeping with the earlier observation<sup>18</sup> that ATP hyperpolarises cells in guinea pig liver slices, presumably by increasing  $P_K$ . The potassium loss was not associated with a change in cell viability. It was greatest with A23187, reaching a maximum of  $23 \pm 2\%$  (s.e.,  $n = 8$ ) of the

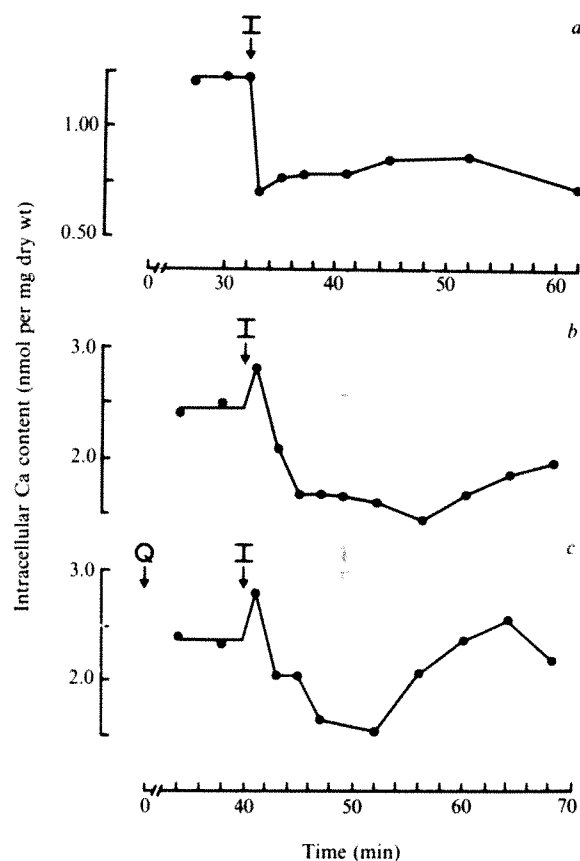


initial cell content (about 290 mmol per kg dry weight) within 1 min when the ionophore was applied at 40–80  $\mu\text{mol}$  per litre of cells, corresponding to an added concentration of 1  $\mu\text{M}$ . With maximal doses of ATP and noradrenaline (100 and 5  $\mu\text{M}$  respectively) the loss was 10% with each agent, again within about a minute. This is of the same order (8%) as seen on exposing guinea pig liver slices to 1  $\mu\text{M}$  noradrenaline<sup>2</sup>. The response to the selective  $\alpha$ -agonist amidephrine was somewhat variable, ranging from 7% to as little as 1% in a few preparations.

The next experiments were prompted by the finding of Armando-Hardy *et al.*<sup>19</sup> that in erythrocytes the influence of cytosolic calcium on  $P_K$  is inhibited by quinine. A similar effect has been observed in barnacle photoreceptors<sup>20</sup>. We might then expect this agent to reduce the effectiveness of the ionophore and of the other agents in inducing K loss from liver cells. Figure 1a shows that this is so; the responses to noradrenaline and amidephrine were also inhibited to a comparable extent. The possibility that quinine was acting by interfering with the primary actions of the ionophore (and, by implication, of the other agents causing potassium loss) was tested by examining the effect of quinine on the  $^{45}\text{Ca}$  movements brought about by A23187. The technique used was based on that described for erythrocytes by Ferreira and Lew<sup>21</sup>, and is described in Fig. 2. This illustrates the effect of A23187 on the  $^{45}\text{Ca}$  content of cells equilibrated with the tracer for 30–40 min, long enough to allow most of the cytosolic calcium to exchange<sup>22</sup>. When the external



**Fig. 1** *a*, Application of A23187 (5  $\mu\text{M}$ , about 5  $\times$  the dose for maximum effect), ATP (100  $\mu\text{M}$ ), noradrenaline (NOR; 5  $\mu\text{M}$  in the presence of propranolol at 5  $\mu\text{M}$ ), and of the selective  $\alpha$ -agonist (–) amidephrine (AM 10  $\mu\text{M}$ ) causes isolated guinea pig hepatocytes to lose potassium. The movement of K between the cells and the incubation medium (Ca, 1.8 mM; Mg, 0.81 mM) was followed by means of a K-sensitive electrode<sup>29</sup> in the cell suspension (which contained ~50 mg cells in 2 ml, and was stirred). A downward deflection indicates an increase in K concentration, corresponding to K loss from the cells: in this experiment, the loss in response to A23187 amounted to 19% of the total cell content. *b*, Quinine (1 mM, applied in 20  $\mu\text{l}$  ethanol, 5 min previously) greatly reduces the K loss initiated by A23187 and by ATP: control responses are from the same batch of cells.



**Fig. 2** Effect of A23187 on the  $^{45}\text{Ca}$  content (nmol per mg dry weight) of guinea pig hepatocytes at two calcium concentrations (*a*, 100  $\mu\text{M}$ , *b* and *c*, 1.8 mM).  $^{45}\text{Ca}$  was included in the suspension fluid from zero time onwards. At the times indicated by the points, 100  $\mu\text{l}$  samples of the cell suspension were placed in 1.5 ml Eppendorf tubes containing 300  $\mu\text{l}$  of *n*-butylphthalate and 200–800  $\mu\text{l}$  of 'washing medium'. This contained NaCl (160 mM), EGTA-NaOH (2 mM, at pH 7.4, to remove extracellularly bound  $^{45}\text{Ca}$ ) and  $^3\text{H}$ -inulin, and was maintained at 0–1  $^{\circ}\text{C}$ . Each sample was immediately centrifuged at 12,000g (Eppendorf centrifuge, Model 5412). The supernatant and the oil were sucked off, and the pellet of cells homogenised in 100  $\mu\text{l}$  distilled water.  $^{45}\text{Ca}$  and  $^3\text{H}$ -inulin were measured by liquid scintillation counting. The amount of labelled inulin gave an estimate of the extracellular fluid trapped in the pellet, and this was used to calculate the  $^{45}\text{Ca}$  content of the cells. A23187 was added (at I) in 20–70  $\mu\text{l}$  absolute alcohol to 15 ml of the cell suspensions. (Final A23187 concentrations: *a*, 10  $\mu\text{M}$ , 690  $\mu\text{mol}$  per litre of cells; *b* and *c*, 2.7  $\mu\text{M}$ , 82  $\mu\text{mol}$  per litre of cells.) *b* and *c* are from the same experiment: in *c*, quinine was added in 15  $\mu\text{l}$  ethanol to a final concentration of 1 mM. Note that quinine does not alter the first two phases of the  $^{45}\text{Ca}$  movement caused by the ionophore.

calcium concentration was 1.8 mM (Fig. 2*b*), the ionophore first caused the  $^{45}\text{Ca}$  content of the cells to increase. This occurred during the initial 15–60 s and was probably a consequence of a net calcium influx generated by the ionophore. A period of calcium extrusion then followed, during which the  $^{45}\text{Ca}$  content fell to below the pre-ionophore level. This second phase has already been reported for rat liver cells<sup>23,24</sup>, as well as for several other tissues<sup>25–28</sup>, and has been attributed to the extrusion from the cells of  $^{45}\text{Ca}$  released from intracellular stores which in spermatozoa have been localised to the mitochondria<sup>26</sup>. In keeping with the idea that internal stores are concerned in liver cells, this phase (and the effect on potassium loss) was unaffected when the external calcium was reduced to 100  $\mu\text{M}$  (Fig. 2*a*). However, the initial increase in  $^{45}\text{Ca}$  could then no longer be distinguished, even when the concentration of ionophore was drastically increased (see Fig. 2*a*). This was presumably because the initial influx of calcium initiated by the ionophore was much

smaller. In high external calcium a third phase of  $^{45}\text{Ca}$  re-accumulation was evident 10–30 min after the ionophore was applied. It was quite variable in both rate and magnitude and will not be discussed here since it occurs at a time when the potassium movements were largely complete.

Figure 2c shows that quinine at the same concentration as had greatly reduced the effects on potassium loss did not interfere with the first two phases of the action of A23187 on calcium exchange. Thus inhibition by quinine of the potassium movement must occur at a later stage, and would seem to be due either to uncoupling of the mechanism by which  $P_K$  is influenced by intracellular calcium, or to blockade of the potassium channels.

A surprising additional observation was that the rapid phase of potassium loss so evident on treating guinea pig hepatocytes with A23187, ATP and  $\alpha$ -agonists was lacking in rat liver cells prepared in exactly the same way. With the rat cells A23187 caused a slow loss of potassium which became appreciable only after several minutes, that is, at a time when the rapid release from guinea pig cells was almost complete. This was not because of a reduced effectiveness of the ionophore since the effect of A23187 on calcium exchange was as great in rat as in guinea pig cells. These findings suggest that the calcium-sensitive potassium channel present in guinea pig liver cells (and, by implication, in those of the toad, rabbit, cat and dog, all of which show hyperkalaemia with adrenaline) is either lacking in rat hepatocytes, or exceptionally insensitive to calcium. That a species difference exists in this regard has been suspected<sup>1,6</sup>, although it is surprising that it should be so striking.

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## Enhancement of Ca spikes in nerve cells of adult mammals during neurite growth in tissue culture

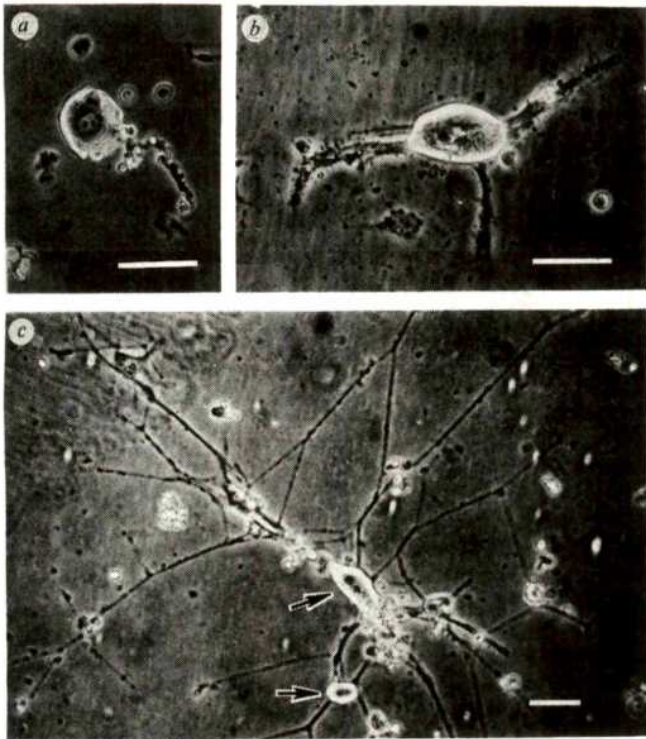
GENERATION of Na and Ca spikes in various types of vertebrate nerve cells<sup>1–6</sup> indicates that both Na and Ca channels are generally involved in inducing action potentials in their cell bodies. Although it has been suggested that these ionic channels may change their configuration in the plasma membrane, in particular during degeneration of nerve cells<sup>7,8</sup> or during growth of axons associated with ontogenetic differentiation<sup>9–11</sup>, no systematic or quantitative study has been reported. We have recently established a technique to obtain primary culture of nerve cells of mature mammals<sup>12,13</sup>, and have also developed a technique to compare membrane capability of carrying inward Na and Ca currents among nerve cells of various ages in the cell culture. We report here that the capability of membranes to carry Ca currents, as represented by the maximum rate of rise (MRR) of a Ca spike, is enhanced during a particular period of the cell culture, whereas there is no significant change in Na currents. This may be explained as an increase in number of the Ca channels or as a change in conformation of the Ca channels, associated, in part, with neurite outgrowth. As we used nerve cells which had fully differentiated in the host bodies, the possibility of an effect due to ontogenetic differentiation in the tissue culture condition<sup>14–19</sup> can be excluded. The present finding also demonstrates another aspect of nerve cell membrane that ionic channel molecules, especially the Ca channels, can alter their features rather flexibly in the plasma membrane.

Dorsal root ganglia (Th<sub>12</sub> to L<sub>5</sub>) were dissected from adult guinea pigs (200–300 g body weight) in a sterile condition, and their nerve cells were dissociated by vibrating the ganglia in collagenase-containing Gey's medium. The nerve cells were collected by weak centrifugation, 1,500 r.p.m. for 5 min. The nerve cells thus obtained (Fig. 1a) were incubated at 37 °C in collagen-coated 3-cm diameter plastic dishes (Nunc, approximately one ganglion per dish) with the tissue culture medium and with sterile air containing 5% CO<sub>2</sub>. The tissue culture medium contained 75% Eagle's minimum essential medium (Microbiological Associates), 15% horse serum (Microbiological Associates) inactivated by heat (60 °C for 1 h), 9% chick embryo extract and 1% penicillin–streptomycin solution (Flow). The medium was changed two or three times a week. So as to obtain steady and compatible cell culture, we carefully kept the cell culture condition uniform, in particular by using the same lot number of dishes, collagen, drugs and some medium components<sup>12,13</sup>.

During the incubation, the nerve cells became attached to the bottom of the dishes in a few hours and then began to extend their neurites gradually in several directions (Fig. 1b). Active growth of the neurites was seen (Fig. 1c) during the first week of cell culture. After about 1 week in the cell culture, fibroblasts began to grow rapidly in number, and occupied most of the available areas of the bottom of the dishes in a few weeks; thus it often became difficult to see the neurites under the phase contrast microscope. The nerve cells, however, survived in the cell culture for more than 2 months<sup>12,13</sup>.

The nerve cell soma was penetrated carefully by a glass microelectrode (20–30 mΩ, filled with 3 M KCl solution) under direct view by inverted phase contrast microscope. Action potentials were elicited in the nerve cells by intracellular passage of current through the recording microelectrode; the membrane potential was determined using a Wheatstone bridge balance. The MRR of the action potential, which was presumed to be proportional to a transient inward membrane current<sup>20</sup>, was measured using an electronic differentiator. The electrophysiological study was done at room temperature, 26–28 °C; pH of





**Fig. 1** Growth of dorsal root ganglion cells of adult guinea pigs in cell culture. *a*, A nerve cell immediately after being dissociated from a ganglion by collagenase treatment. *b*, A nerve cell after 2 days in cell culture. Neurite outgrowth is seen in several directions. *c*, Nerve cells (arrows) after 5 d in cell culture. The length of neurites was often more than 1 mm at this period of the nerve cell culture. Scale bar, 50  $\mu\text{m}$ .

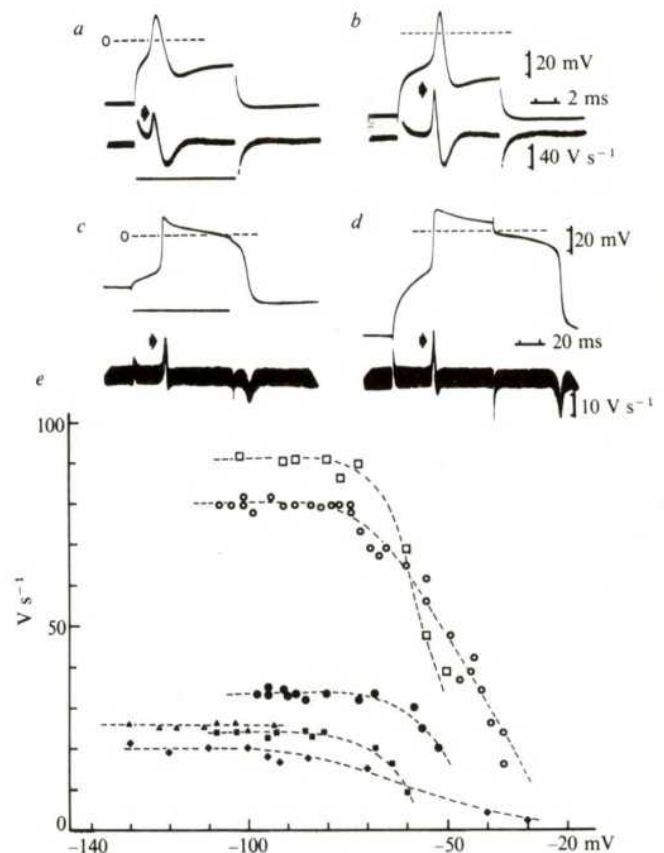
the medium was around 7.4 and was monitored continuously by phenol red.

Figure 2*a* and *b* illustrate spikes elicited in a nerve cell at the resting membrane potential (*a*) and at a hyperpolarised membrane potential (*b*). Judging from their MRRs (arrows in the lower traces), which were proportional to inward membrane currents associated with the respective spikes<sup>20</sup>, the capability of inducing an inward transmembrane current was reduced in the resting membrane (for example, ref. 17) due to a rather small intracellular potential (usually between  $-45\text{ mV}$  and  $-65\text{ mV}$ ). This inactivation was, however, removed by steady hyperpolarisation of the membrane potential below  $-80\text{ mV}$  (Fig. 2*b*: by d.c. current injection), where the MRR became steady at a maximal value which was independent of the membrane potential (Fig. 2*e*, open symbols). We considered this steady maximal value of the MRR as an indicator of the membrane capability of carrying inward current.

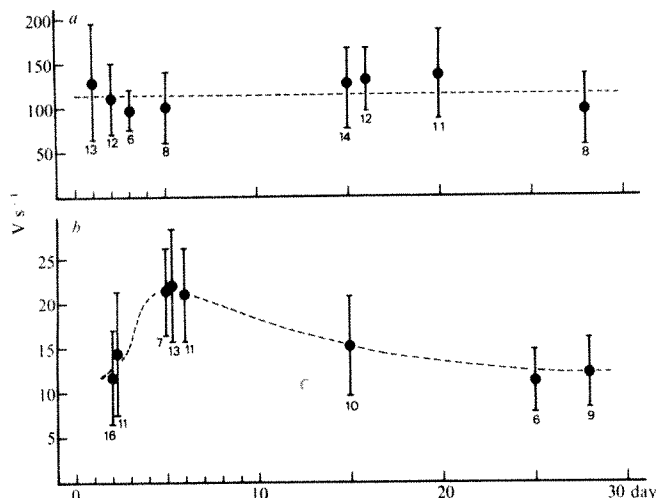
This inward current was carried by  $\text{Na}^+$  as the spike generation was blocked either by externally added tetrodotoxin (TTX,  $1.5\text{ }\mu\text{M}$ ) or by total replacement of external  $\text{Na}^+$  with  $\text{Tris}^+$  (data not shown). Although Ca current was possibly elicited in superimposing the spike potential, its contribution to the MRR above seemed to be very small, because the MRR was obtained within 1 ms after the spike onset (Fig. 2*a*, *b*); this delay was too short to elicit a large Ca current in various excitable membranes<sup>21-23</sup>. Furthermore, the external growth medium contained a small amount of  $\text{Ca}^{2+}$  ( $\sim 1.9\text{ mM}$ ) and no blocker of membrane K conductance (see below), which was not sufficient to induce a large inward Ca current in the nerve cell membrane.

A Ca spike (Fig. 2*c*) was elicited in a nerve cell bathed in a Na-free, tetraethylammonium (TEA)-containing medium;  $\text{TEA}^+$  was added to minimise outward K current<sup>24,25</sup>, so that a small inward current could induce regenerative development of the membrane depolarisation. In addition,  $10\text{ mM Ca}^{2+}$  was

added externally to stabilise intracellular recording and to elicit a large spike potential. We used the Na-free medium described above instead of TTX-containing Na-rich medium because a part of the Na current elicited in tissue-cultured nerve cells is resistant to TTX<sup>10-13</sup>. We considered spikes elicited in the Na-free medium to be due to an inward Ca current for the following reasons<sup>26</sup>: (1) their MRRs were related to external Ca concentration ( $2.5\text{--}10\text{ mM}$ ); (2) they were not blocked by TTX high concentrations ( $3\text{ }\mu\text{M}$ )<sup>12,13</sup>; (3) similar spikes were elicited in Na-free, Ba- or Sr-containing medium<sup>12,13</sup> ( $10\text{ mM}$ , instead of  $\text{Ca}^{2+}$ ); and (4), addition of  $\text{Mg}^{2+}$  ( $40\text{ mM}$ ) or  $\text{Co}^{2+}$  ( $4\text{ mM}$ ) suppressed the spike generation. Although a part of the Ca channel activity was blocked at a small resting membrane



**Fig. 2** Na and Ca spikes, and dependence of their MRRs on intracellular potential. *a* and *b*, Na spikes from a nerve cell after 3 days in cell culture at  $-62\text{ mV}$  (*a*) and  $-89\text{ mV}$  (*b*); the holding membrane potential was changed by d.c. current injection through the recording microelectrode. Bar below the record shows a period when a depolarising pulse current is injected intracellularly. Arrow in the lower trace shows the MRR of the spike. Extracellular solution was the tissue culture medium, which contained about  $141.7\text{ mM Na}^+$ ;  $5.0\text{ mM K}^+$ ;  $1.9\text{ mM Ca}^{2+}$ ;  $0.5\text{ mM Mg}^{2+}$ ;  $119.5\text{ mM Cl}^-$ ;  $25.9\text{ mM HCO}_3^-$ ;  $0.9\text{ mM H}_2\text{PO}_4^-$ ;  $0.5\text{ mM SO}_4^{2-}$ ;  $5.5\text{ mM glucose}$ ;  $1.6\%$  protein, and a small amount of amino acids, vitamins, antibiotics and phenol red; pH of the solution was  $\sim 7.4\text{--}7.6$  during the experiment, and was monitored continuously by colour of phenol red. *c* and *d*, Ca spikes at  $-51\text{ mV}$  (*c*) and  $-100\text{ mV}$  (*d*), elicited in a 5-d nerve cell. Repetition rate of the membrane stimulation was kept at less than 2 to 4 per min. External Na-free medium contained  $133.0\text{ mM Tris-HCl}$ ;  $10.0\text{ mM NaCl}_2$ ;  $10.0\text{ mM TEA-Cl}$ ;  $5.5\text{ mM KCl}$ ;  $5.5\text{ mM glucose}$  and a small amount of phenol red at pH 7.4 (for all Ca spikes in this figure). *e*, Dependence of MRRs of Na and Ca spikes on the holding membrane potential. Different symbols indicate spikes from different cells. Open symbols ( $\circ$  and  $\square$ ) are Na spikes from two nerve cells and filled symbols ( $\bullet$ ,  $\blacktriangle$ ,  $\blacksquare$ ,  $\blacklozenge$ ) are Ca spikes from four nerve cells, respectively. When the membrane potential was held below  $-80\text{ mV}$ , the MRRs of both the Na and Ca spikes were maximal and steady values regardless of the membrane potential.



**Fig. 3** Changes in the membrane capability of initiating inward Na and Ca current during cell culture. *a*, MRRs of Na spikes and *b*, MRRs of Ca spikes in volts per second. Na spikes were elicited in the cell culture medium (see Fig. 2 legend and text). Ca spikes were elicited in Na-free medium containing 63 mM Tris-HCl; 80 mM TEA-Cl; 10 mM  $\text{CaCl}_2$ ; 5.5 mM KCl and 5.5 mM glucose at pH 7.4. Filled circles indicate mean values of the MRRs from nerve cells in dishes of the same lot, and bars are their standard deviations. Numbers of nerve cells sampled are indicated below each point.

potential (Fig. 2c), hyperpolarisation of the membrane potential by d.c. current injection removed this inactivation (Fig. 2d) and the MRR of the Ca spike reached a steady value (Fig. 2e: filled symbols). This value may be considered to represent a capability of carrying the inward Ca current of the neuronal membrane.

The MRRs of the Na and Ca spikes thus obtained were compared for cell cultures of nerve cells of various ages (Fig. 3). The MRRs of the Na spikes did not change appreciably during the 4-week period of cell culture in this study (Fig. 3a). By contrast, the MRRs of the Ca spikes exhibited a temporary increase after about 5 to 6 days in cell culture (Fig. 3b), and thereafter, decayed relatively slowly to a steady level which was close to that obtained at the beginning of the nerve cell culture. This temporary increase by a factor of 1.6, relative to the initial or the later level, is statistically significant ( $P < 0.05$ ). It may be significant that when the MRRs of the Ca spikes are large, the nerve cells seem to be actively extending their neurites (Fig. 1c), suggesting that the increment in the MRRs may be related to neurite outgrowth.

Assuming that capacitance of unit area of the membrane<sup>20</sup> remained unchanged, the temporary increase in the MRRs of the Ca spikes can be interpreted as (1) an increase in the number of the Ca channels in the unit area of the soma membrane; or (2) a conformation change in the Ca channel molecules, which allows entry of a larger amount of  $\text{Ca}^{2+}$ . There are, however, other possibilities such as (3), an increase in the sensitivity of the K current to TEA<sup>+</sup>; a part of the K current which remained in the TEA-containing medium might become smaller during the particular period of the cell culture. All these possibilities reflect a definite change at least in the plasma membrane. A further possibility, (4), is an increment in the electromotive force (e.m.f.) of the Ca current; this seems improbable, because, assuming an intracellular Ca concentration ( $[\text{Ca}^{2+}]_i$ ) ranging between  $10^{-6}$  and  $10^{-8}$  M in the early period of the cell culture<sup>27,28</sup>, a 1.5-fold increment of the e.m.f. can be induced only by a new  $[\text{Ca}^{2+}]_i$  of between  $10^{-8}$  and  $10^{-11}$  M, that is, 1/100 to 1/1,000 of the initial  $[\text{Ca}^{2+}]_i$ .

The physiological functions of Ca spikes recorded from the soma of nerve cells are not as clearly understood as those at presynaptic terminals<sup>29</sup>. Hammerschlag *et al.*<sup>30,31</sup> consider that

amino acid uptake and axonal transportation of proteins are intimately related to Ca entry through the soma membrane. It has also been suggested that Ca entry associated with the firing of nerve cells may be an important part of Ca utilised for axonal transportation<sup>32</sup>. In the present study, active neurite outgrowth was seen at least for several days of an early period of the cell culture (Fig. 1b, c), and simultaneously, enhancement of the MRRs of the Ca spikes was observed (Fig. 3b). During this period of the cell culture, the nerve cells must take up large amounts of nutrients and carry out extensive protein transportation in order to produce new neurites. Ca requirement associated with membrane synthesis and neurite outgrowth may be related to the possible enrichment of the Ca channels in the neuronal membrane.

The reduction of the MRRs of the Ca spikes which was observed in the later period of the cell culture (Fig. 3b) suggests that Ca requirement of the nerve cells may have been reduced for some reason (for example, reduction of neurite growth) or that a membrane part where the Ca channels were populated moved to a position remote from the soma. In the later period, however, observation of the neurites of a nerve cell became difficult because of the rapid proliferation of fibroblasts and the complex interactions of neurites originated from many nerve cells. Further study is thus required to investigate the relationship between the neurite growth and change in the Ca spikes recorded from the soma, especially that in the later period of the nerve cell culture.

Note, however, that there are many other possibilities which could explain the background of the change in the Ca spikes. Whatever the interpretation, it is interesting that ionic channels can alter their distribution or conformation relatively flexibly in the plasma membrane of the mature nerve cells.

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## Neural influence on acetylcholine receptor clusters in embryonic development of skeletal muscles

THE extent to which innervation regulates the embryonic development of skeletal muscle, and the mechanisms of nerve-muscle trophic interactions during development, are little understood. Here we describe studies on the development of skeletal muscles in rat embryos in which all the motoneurons were destroyed, and experiments in which chronic application of tetrodotoxin (TTX) to individual embryos blocked all nerve and muscle electrical activity during neuromuscular junction formation. The results are presented with special emphasis on the regulation of acetylcholine (ACh) receptor distribution. It is already known that ACh receptors in adult tissues are densely clustered under nerve terminals on both muscle<sup>1</sup> and nerve cells<sup>2</sup>. Relatively few receptors are found in extrajunctional regions, and if these are present at all they are uniformly dispersed. Studies of individually teased muscle fibres from rat embryos have shown receptor aggregates (clusters or hotspots) to be present after 16 d gestation<sup>3</sup>, but no more is known about how these clusters are induced to form or to achieve their proper location. Studies of mouse, chick and toad muscles in tissue culture have shown that receptor clusters may form on myotubes that have developed in the absence of nerve tissue<sup>4-6</sup>. In cultured toad muscle these clusters migrate within the muscle membrane to locate beneath nerve terminals<sup>5</sup>. Here, we show that receptor clusters appear on embryonic rat muscle after 15½ d gestation, with striking synchrony in timing, and an ordering of the position of clusters on different fibres within a muscle. These initial events were little changed after irreversible destruction of motoneurons at 14 d gestation except that additional receptor clusters appeared at later times. Paralysis of developing nerve and muscle with TTX did not affect the appearance and progressive increase in size of junctional clusters, but extrajunctional clusters also appeared, as in the denervated muscles. These experiments show that the first appearance of ACh receptor clusters on embryonic skeletal muscle does not depend

on innervation, but that the final ordering of their position on muscle fibres and their later increase in size do require the presence, although not the electrical activity, of the nerve. Electrical activity of nerve and/or muscle is essential to suppress the appearance of extrajunctional receptor clusters, and for the normal progress of muscle growth. This study shows also that mammalian embryos are more accessible to experimental manipulation than may previously have been realised, so that toxins and metabolites may be chronically applied to embryonic tissues *in vivo*, as well as in tissue culture.

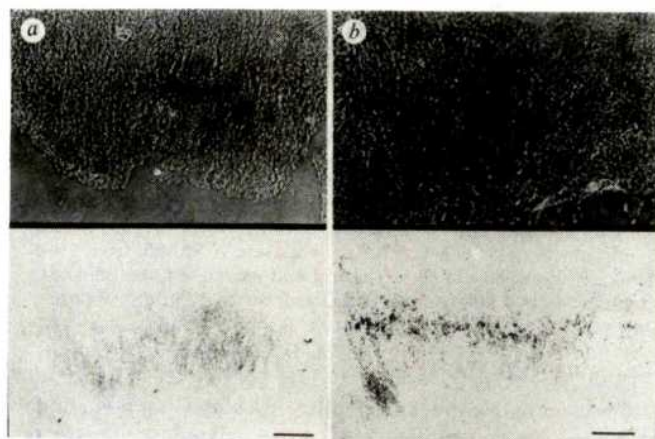
Motoneurons were destroyed by injection of 1 µg of  $\beta$ -bungarotoxin<sup>7</sup> into individual embryos of 14 d gestation or older. Paralysis was not seen in embryos injected at 13 d or earlier. This toxin has been shown to destroy motor nerve terminals<sup>8</sup>, and to cause total loss of motoneurons following injection into chick embryos<sup>9</sup>. We found that a single injection of 1 µg of toxin into individual rat embryos caused a sustained loss of muscle innervation. No recovery of innervation was seen in embryos injected at 14 d and examined at 22 d of gestation. Our evidence for loss of motoneurons in rat embryos includes the absence of nerve fibres in sections of ventral roots examined under the electron microscope, and the absence of large ventral horn neurones in spinal cords of treated embryos. Toxin-treated embryos developed in a grossly normal fashion, although they were lighter in weight than their siblings, and abnormally flexed in posture.

Embryos were paralysed by inserting glass capillaries filled with 1 to 2 µl of 0.03 M TTX into their thoracic or abdominal cavities. One end of each capillary had a pore of 20 µm diameter by 100 µm length to allow slow release of the drug by diffusion. In all cases in which capillaries were fully internalised, embryos remained completely paralysed for at least 3 days, and in control experiments newborn rats were paralysed within 5 min of intra-abdominal insertion of a capillary. Survival rates of 100% were common in groups of embryos treated from 16 d gestation onwards, but implantation of capillaries into 15 d embryos was more difficult.

Clusters of ACh receptors were first seen in normal muscles at 15½ d gestation; before that receptors were diffusely distributed along the developing myotubes (Fig. 1). When first seen, junctional clusters had a mean longest dimension of  $7.84 \pm 0.65$  µm ( $\pm$ s.e.m.). If clusters were present they were seen on the great majority of myotubes within a muscle, indicating that their time of appearance was coordinated throughout the tissue. They were not randomly positioned, but appeared as a band across the midpoint of the developing muscle.

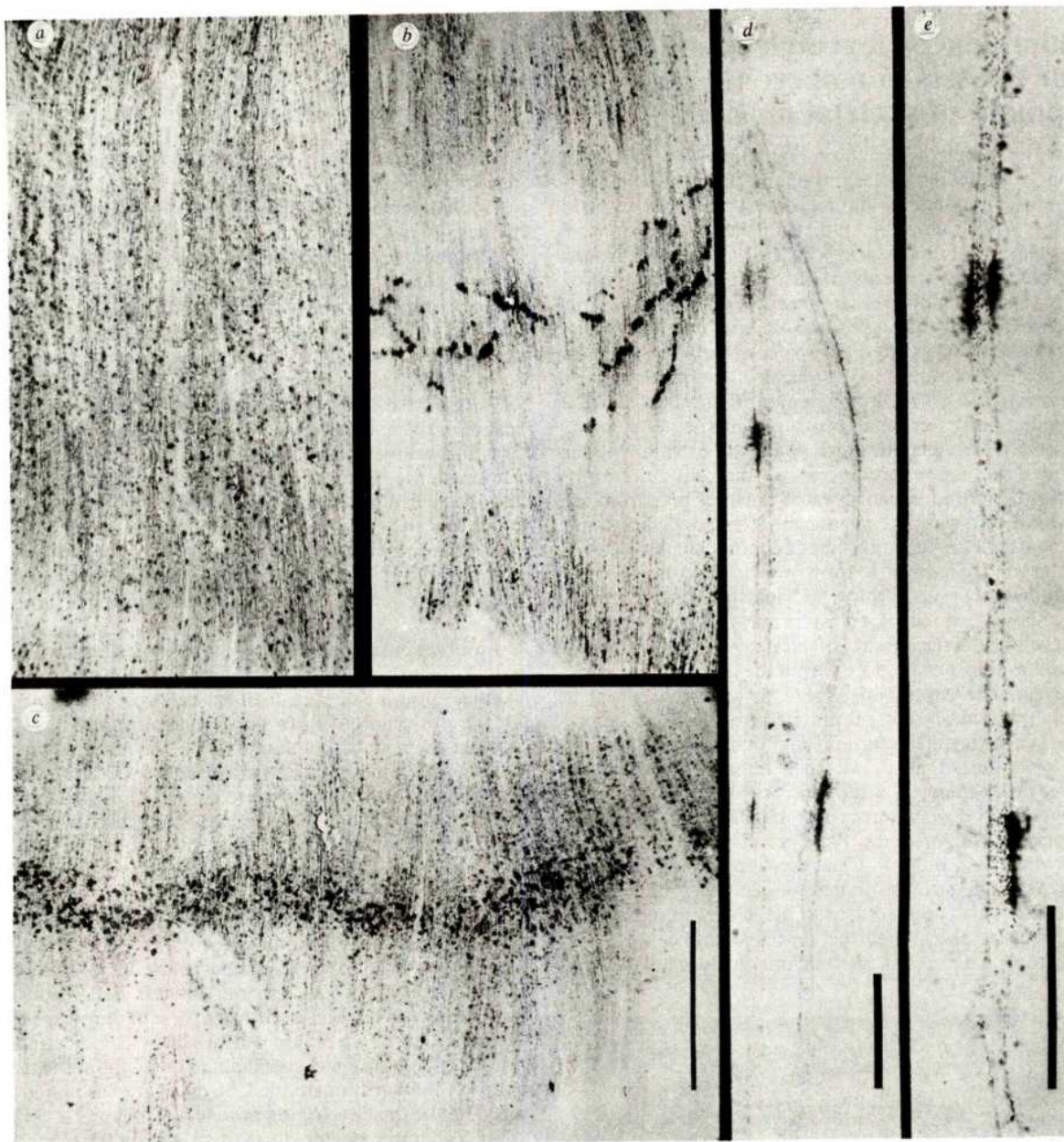
ACh receptor clusters formed on muscle fibres made aneural by injection of  $\beta$ -bungarotoxin at 14 d gestation. Clusters were first seen at 16 d gestation, when they formed a broad band across the middle of the muscle. By 17 d gestation their distribution appeared more widespread than in controls although they did not extend all the way to the tendon ends of muscle fibres. In muscles treated at 14 d and examined at 20 d clusters were randomly positioned along the full length of the muscle (Fig. 2a), with no special accumulation in the middle of the muscle. This is in contrast to normal innervated muscle fibres which have a single cluster near the midpoint of the cell<sup>1,3,10</sup>. Once present, these extrajunctional clusters retained a constant size, with mean maximum dimensions of  $8.97 \pm 0.25$  µm at 16 d, and  $8.96 \pm 0.22$  µm at 20 d gestation. If nerve terminals were destroyed at 17 d and muscles examined after 20 d gestation the endplate clusters remained, and extrajunctional clusters appeared only near the ends of the myotubes, presumably on parts of the muscle that had developed after injection of the toxin. The two types of cluster could be distinguished by their size ( $9.98 \pm 0.26$  and  $6.63 \pm 0.13$  µm) for junctional and extrajunctional clusters, respectively, as well as by their location. Control junctional clusters in untreated littermates at 20 d were  $13.28 \pm 0.29$  µm in length, showing that junctional clusters in the treated muscles ceased their growth on application of toxin.

Paralysis with TTX from 16–19 d or 17–20 d gestation resulted in muscles almost indistinguishable from those treated



**Fig. 1** Autoradiographic demonstration of ACh receptor distribution on early myotubes. *a*, Rat embryo diaphragm muscle after 15 d gestation. Top, phase contrast view of unstained section, showing narrow band of myotubes in the muscle centre, surrounded by myoblasts. Bottom, bright field view to show receptors diffusely scattered over myotubes, but not over myoblasts. No receptor clusters can be resolved at this time. *b*, Muscle after 15½ d gestation, illustrating formation of receptor clusters. Scale bars, 100 µm. Embryos were dated by taking 9 am on the morning when a copulation plug was seen as time zero. Embryos were examined between 9 and 11 am (d), or between 7 and 11 pm (½). Receptor localisation was mapped by incubating muscles with <sup>125</sup>I- $\alpha$ -Naja toxin, washing overnight, fixing in glutaraldehyde-paraformaldehyde, making 20-µm frozen sections, and exposing autoradiographs at 4 °C for 3 days.





**Fig. 2** Autoradiographic demonstration of ACh receptor distribution on embryonic rat diaphragm muscles after denervation or paralysis with TTX. *a*, Aneural muscle from embryo after 20-d gestation injected with  $\beta$ -bungarotoxin at 14 d. Receptor clusters are scattered randomly from end to end of the muscle, and no endplate zone can be distinguished. *b*, Muscle from 20-d embryo following paralysis with TTX from 17 d onwards. Endplate clusters are present near the centre of the muscle, with extrajunctional clusters near the fibre ends. *c*, Muscle from 19-d embryo following paralysis with TTX from 16 d onwards. There is no clear area between endplate clusters and extrajunctional clusters. Individual silver grains cannot clearly be resolved in these low-power micrographs (scale bar, 500  $\mu$ m). *d* And *e*, receptor distribution on muscle fibres teased from 16 d–19 d TTX-treated muscle, to show that multiple clusters were present along the length of single muscle fibres. Scale bars 50  $\mu$ m. Pictures similar to those in *b*–*e* were obtained from muscles after  $\beta$ -bungarotoxin injections at 17 or 16 d, respectively.

with  $\beta$ -bungarotoxin at the same initial times. Paralysis during the period 16–19 d produced muscles in which junctional clusters could clearly be distinguished from extrajunctional clusters (Fig. 2*c*), but with no cluster-free regions of muscle membrane. Paralysis from 17–20 d gave rise to muscles with a clear area containing only diffusely distributed receptors between the junctional and extrajunctional clusters (Fig. 2*b*). The only distinction to be made between  $\beta$ -bungarotoxin-treated and TTX-treated muscles was in the size of the junctional clusters; for example 17–20 d TTX-treated muscles had junctional clusters with mean dimensions of  $13.98 \pm 0.34 \mu$ m, similar to those of 20 d controls. Extrajunctional clusters were  $7.46 \pm 0.14 \mu$ m in length.

Paralysis with TTX from 15–18 d produced muscles less comparable with  $\beta$ -bungarotoxin-treated tissues. The one control (water-filled capillary) embryo which survived from this

time was normal with respect both to gross development and to ACh receptor distribution, indicating that effects on treated embryos were due to the TTX and not to insertion of the capillary. Of three treated embryos which so far have survived, one was dwarfed and two appeared normal when examined at 18 d gestation. Muscle development was severely retarded, with myotubes only in the centre of the diaphragm muscles, surrounded by large areas of unfused myoblasts. After the TTX had been washed off, isolated muscles contracted in response to nerve stimulation. Junctional clusters of normal size ( $13.70 \pm 0.33 \mu$ m) were present, but there were few extrajunctional clusters.

Diaphragm muscles contracted in response to nerve stimulation at 15-d gestation, before clusters of ACh receptors could be resolved with our techniques. Intracellular recording from 15-d intercostal muscles (M. J. Dennis and A.J.H., unpublished)



showed that nerve stimulation produced endplate potentials in every muscle fibre tested (50/50), and that miniature endplate potentials at this age were much smaller than in 16-d muscles, despite similar resting potentials ( $>70$  mV) at both times. Nerve fibres are physically present in developing muscles before fusion of myoblasts into myotubes has begun<sup>11</sup>. Thus muscles contract in response to nerve impulses at least 6 h before ACh receptor clusters can be resolved. It is our impression that clusters first appear as distinct units, rather than growing by progressive aggregation of dispersed receptors. It is interesting to note that once an area of muscle has developed without receptor clusters they cannot appear there later, so that denervation leads only to an increase in the density of dispersed receptors in that region. That this is not simply a matter of time was shown by denervating rat intercostal muscles at birth, and examining muscles in which denervation was maintained for up to 4 weeks; extra-junctional clusters appeared only near the tendon ends of the muscle fibres, in regions which had developed since the time of denervation.

Neither destruction of the innervation nor paralysis of nerves and muscles in rat embryos prevented the formation of receptor clusters, and fusion of myoblasts into myotubes was only retarded and not arrested by these procedures. We conclude that, by evoking muscle contraction, innervation speeds the expression of a developmental programme inherent in myogenic cells for elongation and growth of myotubes and for the first appearance of ACh receptor clusters. The effects of electrical stimulation or of application of TTX to spontaneously contracting monocultures of myotubes in tissue culture are consistent with this description<sup>4</sup>. A specific inductive action<sup>12</sup> of the nerve terminal, exerted independently of electrical activity, must be postulated to regulate the positioning of the cluster under the nerve terminal<sup>5,13</sup> and for its progressive increase in size<sup>3</sup>. In the presence of a nerve terminal electrical activation of the muscle prevents the appearance of further receptor aggregates. TTX treatment over the period 15–18 d had an effect more drastic than denervation, perhaps by affecting spontaneous as well as nerve-induced muscle contractions, and so retarding muscle growth. If the appearance of extra-junctional clusters in denervated muscles is secondary to muscle growth, as seems likely (Fig. 2b), their apparently paradoxical absence from the 15–18-d treated muscles may be explained by the muscles being in a developmental stage reached by  $\beta$ -bungarotoxin-treated tissues at 16½ or 17 d. Further experiments are needed to clarify this point.

The only known action of TTX is to block the action potential mechanism in excitable tissues. Chronic local application to mature motor nerves causes prolonged paralysis but only partially blocks the trophic effect of the nerve on muscles<sup>14,15</sup>. The residual trophic action of the paralysed nerve may depend on materials carried by axonal transport<sup>16</sup>, although this is debatable<sup>17</sup>. Electrical stimulation of adult motoneurons increases the amount of proteins carried by axonal transport<sup>18</sup>, and as application of TTX to embryos blocked action potentials in motoneurone cell bodies as well as axons this might have reduced the rate of synthesis of the hypothetical trophic substances. Thus the argument made above for electrical activation being of primary significance in nervous regulation of embryonic muscle development is the most economical, but it is not definitive.

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## Composition and control of secretions from tracheal bronchial submucosal glands

TRACHEAL bronchial submucosal (TBS) glands are thought to supply a significant fraction of respiratory tract fluid (RTF)<sup>1</sup>, which is critically important in cleaning the airways of the lung. In the cleaning process, cilia in the tracheal bronchial tree continuously sweep the RTF, with debris, out of the lung. But abnormalities in the physicochemical properties of the RTF may impair this clearance, resulting in fluid stagnation, infection and obstruction of the airways. Unfortunately, because of the small size and inaccessibility of TBS glands and the difficulty in collecting their uncontaminated secretions, little is known about their physiology. We have developed methods for collecting and analysing secretions from single TBS glands of the cat *in vitro*, and we describe here the composition and control of the fluid secreted by these glands. We have found that the composition of the secreted fluids is very similar to that of the extracellular fluid and that secretion flow rates are influenced predominantly by  $\alpha$ -adrenergic and cholinergic agonists. In view of the well known exocrinopathy in the lethal genetic disease cystic fibrosis (CF)<sup>2</sup>, we believe these findings to have implications for pathological changes in the airways in this disease.

Tracheae were excised from seven mongrel cats anaesthetised with pentobarbital (35 mg per kg). Using a modification of a technique<sup>3</sup> for observing secretion *in vivo*, the tracheae were opened along the dorsal fold, and the epithelial surface was dried quickly with cotton sponges and a jet of freon-13 gas. The surface was coated immediately with water-saturated paraffin oil, and a segment of the trachea (about 1 cm<sup>2</sup>) was mounted in a chamber such that the serosal tissues were constantly bathed in Ringer solution which mimicked interstitial fluid (Table 1), while the epithelial surface remained covered with oil. Secretion was stimulated by adding appropriate concentrations of bethanechol (cholinergic agonist), methoxamine ( $\alpha$ -adrenergic agonist) and isoprenaline ( $\beta$ -adrenergic agonist) to the bath. Under a dissecting microscope, small droplets of secretory fluid were observed to form on the tracheal epithelial surface shortly after stimulation. Timed collections of droplets secreted from three to four glands were taken up between oil blocks in constant-bore capillaries (78  $\mu$ m inner diameter), and droplet volumes were measured for rate determinations usually over a period of about 5 min. The elemental chemical composition of each sample was assayed by microdroplet X-ray analysis for Na, K, Ca, Cl, S, and P (ref. 4).

Secretory rates reached maximal values about 10 min after the application of agonists, after which the rates generally declined (Fig. 1). Maximal rates as high as 20 nl per min per gland were induced but not sustained by bethanechol or methoxamine. Maximal secretory rates with  $10^{-5}$  M isoprenaline in the bath were generally less than 10% of the maximal rate induced by  $10^{-6}$  M bethanechol or  $10^{-5}$  M methoxamine (Fig. 1). Glands generally responded to stimulation for at least 5 h *in vitro*, but, probably due to variation in gland sizes, there was considerable variation in secretory rates between glands.

The composition of secretions was basically the same as that of the bath. There were no significant changes in the concentrations of any of the principal electrolytes (Na, Cl, K or Ca) as a function of the secretory rate or the nature of the applied stimulant (Table 1). Hence, if TBS glands normally secrete essentially isotonic fluids *in vivo* we conclude that, unlike most other exocrine glands, the cat TBS gland does not significantly modify the electrolyte composition of the primary fluid in the ductal region before it is excreted.

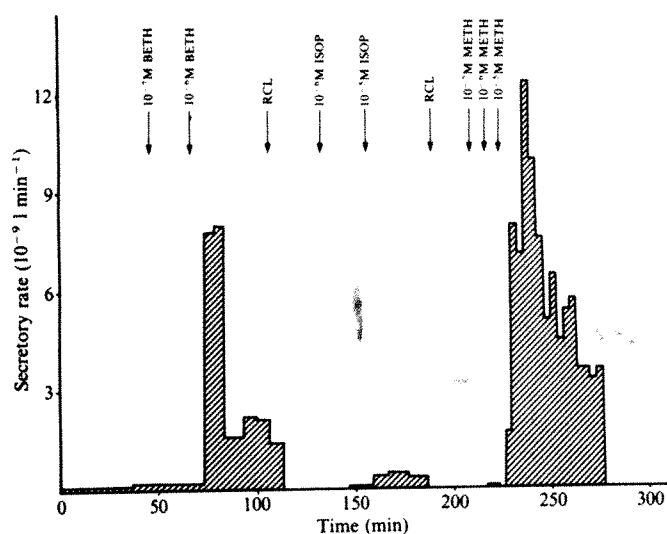


Fig. 1 A typical secretory rate response (ordinate) of a single TBS gland to increasing concentrations of bethanechol (BETH), isoprenaline (ISOP) and methoxamine (METH) in the bathing media. The time of addition of each drug at the indicated concentration is shown by the position of the inverted arrow relative to the abscissa. Rinsing was carried out by changing the bath to Ringer's solution (RCL). Concentrations, an order of magnitude higher than those shown, were examined in trials, but did not reveal noticeably higher maximal rates, even though rinsing times were increased.

However, a notable exception to this otherwise relatively constant composition is in the mean concentration of sulphur, which varied significantly depending on the nature of the stimulating agent. Methoxamine-stimulated secretions contained significantly less S (2.1 mM) than either bethanechol (3.2 mM;  $P < 0.025$ ) or isoprenaline (4.8 mM;  $P < 0.005$ ) stimulated secretions, but the difference between the mean concentration of S in bethanechol and isoprenaline stimulated secretions was not statistically significant ( $P < 0.1$ ). Sulphur concentration varied widely among glands (range 1–16 mM) and was inversely correlated with secretory rate (for individual glands, correlation coefficients ranged from  $-0.33$  to  $-0.82$ ).

Both the wide variation in the S concentration and the negative correlation with secretory rate indicate that S appears in the gland secretion by a route different from that of elements in the precursor fluid. Because isotopic S is taken up by TBS gland cells<sup>5</sup> and incorporated into the glycoprotein component of tracheal secretions<sup>6</sup>, mucus is the most probable additional source of S. If the concentration of S reflects the mucus content of the secretions, the data indicate that isoprenaline stimulates secretions with low flow rates but relatively high mucus content,

Table 1 Concentrations (mM) of the major inorganic elements in TBS gland secretions after stimulation with  $\alpha$ - and  $\beta$ -adrenergic and cholinergic agonists

	Na	Cl	K	Ca	P	S
Methoxamine ( $\alpha$ -adrenergic) $n \geq 35$	135 $\pm$ 1.7	119 $\pm$ 1.7	5.7 $\pm$ 0.18	0.6 $\pm$ 0.06	0.9 $\pm$ 0.11	2.1 $\pm$ 0.27
Bethanechol (cholinergic) $n \geq 13$	134 $\pm$ 2.1	124 $\pm$ 1.9	6.3 $\pm$ 0.28	0.7 $\pm$ 0.12	1.3 $\pm$ 0.14	3.2 $\pm$ 0.4
Isoprenaline ( $\beta$ -adrenergic) $n \geq 13$	141 $\pm$ 2.7	126 $\pm$ 2.5	5.7 $\pm$ 0.30	0.6 $\pm$ 0.08	1.1 $\pm$ 0.18	4.8 $\pm$ 1.09
Incubation	136	119	5.0	2.0	1.2	1.2

Except for S there was no statistically significant difference in the concentration of any element among secretions stimulated with the different agonists. Sulphur in methoxamine-stimulated secretions was significantly lower than S concentrations in the other two groups of secretions as determined by Student's *t*-test<sup>12</sup>. Errors are standard errors of the mean; the number of samples analysed was equal to or greater than *n*.

whereas methoxamine stimulates secretions of high flow rates but relatively low mucus content. Bethanechol stimulates secretions of both high flow rates and relatively high mucus content (Table 1, Fig. 1). In other exocrine glands such as the parotid, protein secretion is higher during  $\beta$ -adrenergic stimulation while flow rates are higher during  $\alpha$ -adrenergic stimulation<sup>7</sup>. Similarly, the rates and protein content are higher with cholinomimetic stimulation in the salivary gland, but the higher protein content may be due to  $\beta$ -adrenergic effects of the cholinergic agonist<sup>8</sup>.

The observation that different stimulatory drugs evoked distinct maximal secretory rates and different S (mucus) concentrations supports the view that the physical properties of the RTF may be regulated by combined sympathetic and parasympathetic controls<sup>5</sup>. If the autonomic nervous system controls the relative amounts of fluid and mucus, autonomic disturbances in CF<sup>9,10</sup> may produce abnormal volumes of secretions or abnormal concentrations of mucus which depress respiratory clearance mechanisms. This type of defect may explain why, although widely accepted, no defect in mucus production in this disease has been established<sup>11</sup>. Also, if there is no ductal modification of the precursor fluid, the defect in CF would seem to be basically a problem of secreting appropriate volumes and mucus concentrations.

In conclusion, cat TBS glands produce a secretion which is essentially isotonic, but which varies in S and possibly mucus content as a function of the nature or degree of stimulation. The results are consistent with the possibility that malfunctions in the TBS glands in CF may alter the properties of the RTF and thereby impair airway clearance, rendering the CF lung highly susceptible to infection and obstruction.

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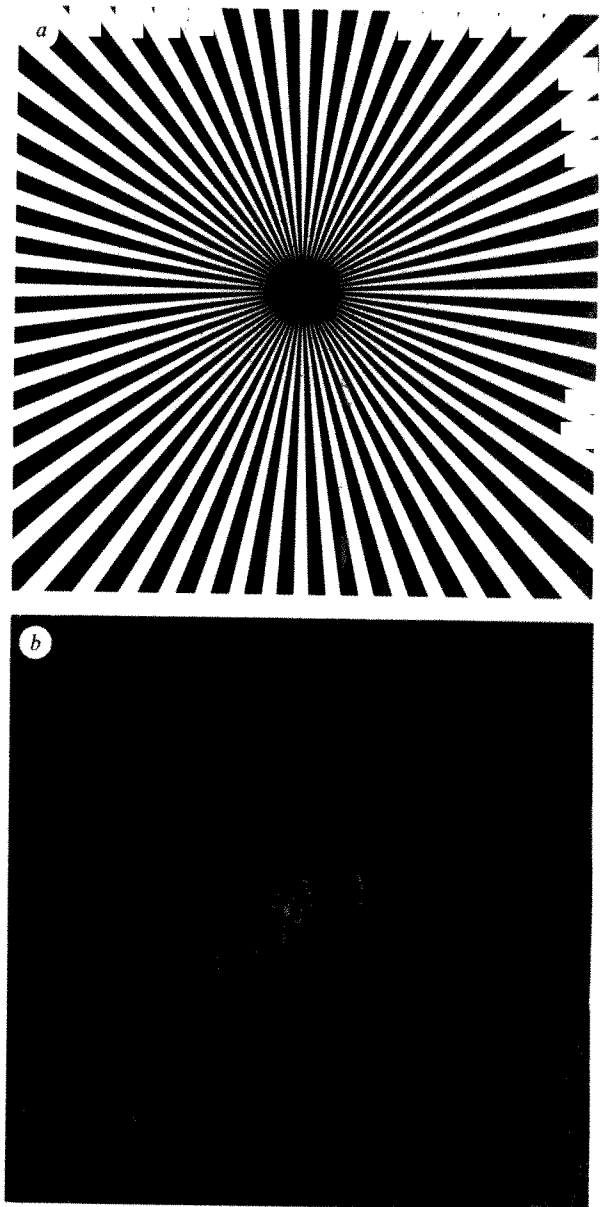
## Clues to the site of origin of the complementary image

PATTERNS of near-parallel black-and-white lines induce an anomalous state of the visual system, in which a superimposed test field of dynamic noise (such as the 'snowstorm' on a detuned TV receiver) seems to stream in directions roughly perpendicular to the inducing contours<sup>1</sup>. The illusory streamers form a 'complementary image' (CI) which for the stimulus of Fig. 1a comprises a family of wavy circles, as sketched by J. P. Wilson<sup>2</sup> in Fig. 1b. When two orthogonal gratings are superimposed, the streamers lie at 45° to both, as if the resulting bias obeys the rule of vector summation<sup>3</sup>. J. P. Wilson<sup>4</sup> has observed rudimentary signs of directional bias and streaming even at a single black/white contour; and M. E. Wilson<sup>5</sup>, using only simple pairs of test spots illuminated in sequence, found that the illusory 'phi' motion seen between the spots was enhanced for directions orthogonal to the contours of a superimposed grating. When the inducing pattern is presented to one eye and the noise field to the other, however, little or no CI is observed<sup>1,6</sup>. It has seemed logical<sup>1</sup> to attribute these phenomena to some form of adaptive cooperative activity among orientation-sensitive cells of the visual system, leading to a kind of 'simultaneous contrast' in the orientation domain, that is, a bias of the signalling system for motion and contour orientation in favour of the orientation(s) not present in the inducing stimulus.

Human observers can perceive contours outlined only by contrasts of texture or colour almost as readily as those due to differences in luminance. It would therefore help in locating the site of origin of the CI to know whether the system responsible can be directionally biased by patterns outlined only by contrasts of texture or colour. Hammond and I<sup>7,8</sup> have recently found that 'simple' cells in area 17 of cats, which respond briskly to a suitably orientated moving black bar, show no response to a similar (clearly recognisable) bar of random visual texture moving over a static textured background of the same mean luminance. Although the colour sensitivity of simple cells in area 17 of primates is still controversial<sup>9,10</sup>, it seems that most are also insensitive to boundaries outlined by colour contrast alone. Thus, if the CI could be induced by patterns of texture or colour contrast alone, this would suggest that it originated in a system not dependent on simple cells for its input (unless human simple cells differed in this respect from those of cat and monkey). If, however, induction of the CI required the patterns to be outlined by luminance contrast, this would point to the simple-cell system as the likely site of origin.

In the first experiment, a 60-ray pattern 12 cm in diameter, similar to Fig. 1a, was cut from white paper and placed over the screen of a detuned TV receiver generating dynamic visual noise. The paper pattern was front-lit at a luminance of 30 cd m<sup>-2</sup>. With the mean luminance of the TV display either higher or lower than that of the paper pattern, the normal CI was clearly visible in the noise. When the mean luminance was adjusted to the same level (30 cd m<sup>-2</sup>), however, the CI disappeared, although the white ray pattern remained clearly perceptible by contrast with the dynamic noise between its spokes. (Some 60 observers have confirmed these findings).

An analogous experiment was then carried out using patterns outlined by colour contrast. A pair of conventional 100-W slide projectors with red and green filters (Cinemoid nos 14 and 24) were used in conjunction with a mixer cube to project on to the noise-filled TV screen the same 60-ray figure outlined at ~20 cd m<sup>-2</sup> in red and green. The mean luminance of the (white) noise field alone was adjustable for optimum effect. When the luminances of the interleaved red and green rays were matched the CI was absent or much reduced for all of five observers, although with the red or green rays alone (that is, one projector switched off) it was clearly visible.



**Fig. 1** a, The stimulus used for the production of a CI. b, The family of wavy circles obtained.

It therefore seems that the anomalous physiological condition responsible for the CI arises not at the level of the pattern-recognition system *per se*, but at an earlier stage of visual information processing which is specifically sensitive to contrasts of luminance, although not of texture or (perhaps) colour. The absence of significant interocular transfer suggests a location in the monocular visual sub-system. The parallel with the preferences shown by simple cells in area 17 of cats and monkeys points to these cells, or others dependent on them, as likely candidates.

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## Contribution to reproductive effort by photosynthesis of flowers and fruits

REPRODUCTION is often a lethal or semi-lethal activity, and for iteroparous plants it is often possible to show that reproduction has costs that are expressed in a reduced growth rate and/or an increased death rate<sup>1</sup>. Attempts have been made to compare life history patterns in flowering plants by measuring the fraction of a plant's annual dry matter production (or calorific value) that is allocated to reproduction (for example refs 2–4). The assumption is that the reproductive parts represent a cost in energy or materials. Clearly mineral nutrients and water must be gained by the reproductive structures from the remainder of the plant, but this is not necessarily true for the energetic and carbon economy of the reproductive structures. Many flowers and fruits are green and a fraction of the energy and carbon might be obtained by direct photosynthesis within these structures. This might be especially important during embryo growth if carpels and other organs that remain attached after flowering are green. In such cases the conventional estimation of reproductive effort (dividing seed weight by plant weight) would be incorrect and comparison of life history patterns and their evolutionary meaning would be invalid. There are reports of significant contributions of *in situ* photosynthesis in flower and seeds to their growth. Biscoe *et al.* have estimated that 47% of the carbon required for seed production in barley is provided by photosynthesis of reproductive and immediately adjacent plant structures<sup>5</sup>. Bazzaz and Carlson have shown that in the annual weed *Ambrosia trifida* L., net photosynthesis by reproductive structures amounts to 41 and 51%, respectively, of the carbon required for male and female inflorescences<sup>6</sup>. Here we report an analysis of the carbon budget of reproduction for 15 temperate deciduous trees. The budget was determined by measuring the weight, photosynthesis and dark respiration of flowers or inflorescences from bud break until seed maturity.

Inflorescences, flowers or fruits with a 3–5-cm piece of stem were excised from plants, sealed in vials containing water, and the rate of CO<sub>2</sub> exchange of the inflorescence was determined, first in the light (700 µE m<sup>-2</sup> s<sup>-1</sup>) and then in the dark

(1 µE m<sup>-2</sup> s<sup>-1</sup>) (ref. 7). Carbon dioxide exchange was measured at an air temperature equal to the mean daily maximum temperature (calculated from 20-yr summaries of weather data for Morrow Plot, Urbana, Illinois) for each day in which measurements were taken.

The data for photosynthetic and dark respiration rates were fitted by least squares regression to exponential, logarithmic and power univariate expressions with time as the independent variable. The expression with the largest coefficient of determination (*r*<sup>2</sup>) was chosen to represent the rate of CO<sub>2</sub> exchange throughout reproductive development.

Weight data were fitted to a linear form of two different functions and the one with the highest *r*<sup>2</sup> was chosen to represent weight gain for that species. One of the functions used had both a lower and an upper asymptote

$$W = \frac{K}{1 + e^{c-bt}} \quad (1)$$

and the other had only an upper asymptote

$$W = K(1 - e^{c-bt}) \quad (2)$$

where *W* = weight of flower or inflorescence in mg, *K* = weight at maturity in mg, *e* = base of natural logarithms, *c* = a constant of integration determining the position of the curve relative to the origin, *b* = the capacity for increase in units of mg/t, and *t* = time in days since 14 March. In fitting data to equations (1) and (2), *K* was estimated by trial and error to maximise *r*<sup>2</sup>. A carbon budget was constructed as described previously<sup>6</sup>.

There were three general types of weight gain curve for reproductive structures (Fig. 1). A continuous form of equation (1) represented the best expression for growth in 11 species (Table 1) and is exemplified by *Platanus occidentalis*. Equation (2) was the best expression for reproductive growth for two species represented by *Acer saccharum* (Fig. 1b). The third type of reproductive growth is the split or two-phase form of equation (1) as found for *Liriodendron tulipifera* and *Magnolia stellata* (Fig. 1a). Large showy petals were formed during the first part of reproductive development but fell off by about day 65, causing a sharp decrease in weight. Other species also lost some floral parts during development but that did not alter the smooth character of the growth curve.

The pattern of development described by equations (1) and (2) may reflect important differences in the metabolic cost of

**Table 1** Floral sexuality, weight gain, photosynthesis and respiration, and percentage contribution to reproductive effort of ♀ flowers and seeds of selected species

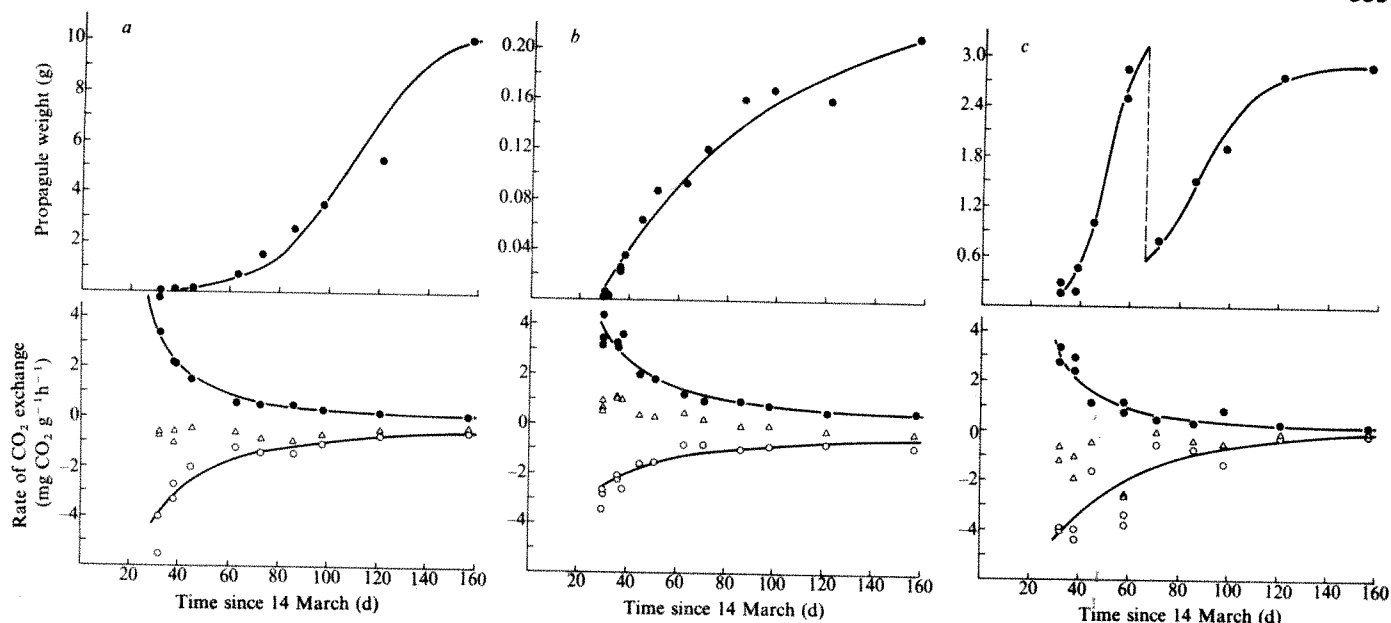
	Floral sexuality	Overdry weight at maturity (mg)	Totals GP (mg)*	Total daytime respiration (mg)*	Total night-time respiration (mg)*	Total respiration 3+4 (mg)*	Net photosynthesis 2-5 (mg)*	% Gain or % loss (2-5)/(100) †	% Contribution GP to total carbon balance 2/(1+5) ‡	Coefficient of determination ( <i>r</i> <sup>2</sup> )	
										Type 1 curve	Type 2 curve
<i>Acer platanoides</i>	H	254	312	156	74	230	82	32.3	64.5	0.894	0.980
<i>Tilia platyphyllos fastigiata</i>	H	1,868	794	283	124	407	387	20.7	34.9	0.902	0.721
<i>Acer rubrum</i>	H	28	15	13	6.8	20	-4.8	-17.1	32.9	0.946	0.922
<i>Acer saccharum</i>	H	210	132	141	47	188	-56	-26.7	33.2	0.860	0.960
<i>Magnolia stellata</i>	H	823	340	247	119	366	-26	-3.2	28.4	0.971	0.835
<i>Cercis canadensis</i>	H	208	96	93	44	137	-41	-19.7	27.9	0.961	0.958
<i>Liquidambar styraciflua</i>	M	2,375	955	1,103	510	1,613	-658	-27.7	23.9	0.999	0.945
<i>Betula pendula</i>	M	565	192	221	101	322	-130	-23.0	21.7	0.910	0.972
<i>Celtis occidentalis</i>	H	342	118	139	65	204	-86	-25.1	21.5	0.908	0.994
<i>Prunus serotina</i>	H	131	38	46	21	67	-29	-22.1	19.2	0.959	0.937
<i>Liriodendron tulipifera</i>	H	2,900	1,032	1,970	914	2,884	-1,852	-63.9	11.5§	§	0.975
<i>Ulmus americana</i>	H	7.5	0.83	0.86	0.50	1.4	-0.53	-7.1	9.3	0.999	0.989
<i>Carya ovata</i>	M	8,135	944	2,367	1,068	3,435	-2,491	-30.6	8.2	0.995	0.848
<i>Platanus occidentalis</i>	M	10,158	1,072	3,540	1,699	5,239	-4,167	-41.0	7.0	0.979	0.903
<i>Quercus macrocarpa</i>	M	3,042	104	1,008	512	1,520	-1,416	-46.5	2.3	0.966	0.685

\* H, hermaphrodite; M, monoecious; GP, gross photosynthesis units of C<sub>6</sub>H<sub>10</sub>O<sub>5</sub>.

† Gain or loss due to CO<sub>2</sub> exchange of propagule expressed as a percentage of propagule weight.

‡ Gross photosynthesis of propagule expressed as a percent of the total amount of carbon for production of a mature propagule (2/1+5 × 100).

§ Contribution based on two parts of weight gain curves in Fig. 3, *r*<sup>2</sup> values were 0.931 for the first part and 0.993 for the second.



**Fig. 1** Increase in growth and changes in the rates of gross photosynthesis (●), dark respiration (○) and net photosynthesis (Δ) in flowers and fruits of *Platanus occidentalis* (a), *Acer saccharum* (b) and *Liriodendron tulipifera* (c).

reproduction. Systems that are well described by equation (1) apparently lack an initial mass transfer of carbohydrate from the plant into the developing reproductive structure but those described by equation (2) seem to have such a transfer.

The amount of carbon supplied from *in situ* photosynthesis to the total carbon required for production of mature seed varied from 64.5% for *Acer platanoides* to 2.3% for *Quercus macrocarpa*.

The results suggest that flowers and fruits should not be regarded simply as a cost to the carbon budget of the rest of the plant. Clearly in some cases green flowers may be sufficiently active photosynthetically to pay a major fraction of the energetic and carbon cost of their own structure and that of the ripened seed. Thus it is misleading to express reproductive yield as if it is the product of leaf photosynthesis. One implication of this finding is that the energetic costs to an animal-pollinated plant with showy non-green flowers may be two-fold—both the carbon cost of making the structure and the costs of sacrificing its potential photosynthetic ability. The sharp break in the floral growth curves of the *Liriodendron*-type emphasises this point. Furthermore, in the improvement of crop plants, we should perhaps consider the extent to which the photosynthetic activity of floral structures might be improved.

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## Is compensatory growth a complicating factor in mouse teratology?

METHODS developed in an attempt to optimise the chances of identifying a teratogen generally involve the administration of the compound to a pregnant female in a number of ways, followed by autopsy about 24 h before parturition. This system maximises the period during which abnormalities may develop and circumvents loss by cannibalisation of dead or deformed newborn animals by their mother. The methodology is simple and efficient but provides no information about the immediate response of the embryo to the drug and any capability it may have for regulation or recovery from damage. Thus developmental disturbances could pass unnoticed. However, the damage, for example behavioural or reproductive abnormality, may manifest itself postnatally, and this has recently aroused some concern<sup>1,2</sup>. We report here on the mode of action of a known teratogen, mitomycin C (MMC) on mouse embryos. In rodents this antibiotic is teratogenic at high doses. In rats the LD<sub>50</sub> and teratogenic dose seem to coincide, but for mice these figures may differ considerably<sup>3–5</sup>. Abnormalities reported include exencephaly, spina bifida<sup>6</sup>, skeletal and other non-nervous system defects<sup>7</sup>. The frequency of abnormality and dose response is strain dependent in mice. We know of no reports on the immediate response of mouse embryos to MMC and of only one on the postnatal development of mice exposed to the drug *in utero* in mid-gestation<sup>8</sup>. Our results indicate that, during early organogenesis stages, the mouse embryo has a remarkable capacity for recovery from severe damage and developmental retardation induced during primitive-streak stages, when drugs were thought more likely to be lethal than teratogenic.

Random bred Q strain mice were used. Pregnant females received a single injection of MMC intraperitoneally 6½ or 7 d postcoitum (p.c.). Two doses were used, 100 and 150 µg per mouse (~4 and 6 mg per kg). There were no noticeable differences in response to the two doses, so data have been pooled. In this strain the primitive streak begins to form between 6½ and 6¾ d p.c. Initially autopsies were done at 7½ and 8½ d p.c.

**Table 1** Cell numbers and mitotic activity in control and MMC-treated 7½-d embryos

<i>n</i>		Control 6	MMC 5
No. of cells	Ectoderm	7,618 ± 443	978 ± 157
	Mesoderm	3,743 ± 256	374 ± 53
	Endoderm	1,019 ± 57	374 ± 33
M/A index	Ectoderm	3.9 ± 0.2	12.5 ± 1.5
	Mesoderm	1.9 ± 0.2	5.2 ± 0.7
	Endoderm	4.5 ± 0.6	6.1 ± 0.7

Camera lucida drawings were made of transverse sections and tissue volumes computed from planimeter measurements of tissue areas. Cell numbers were then calculated from a knowledge of cell volume<sup>13</sup>.

but were subsequently extended to various other time points, including postnatal development.

At 7½ d MMC embryos were morphologically normal but very small (Fig. 1a, b). On the other hand, the extra-embryonic regions of the egg cylinder are of normal size, but in some the mesodermal layer of the exo-coelom is incomplete. Serial reconstructions of embryos show that in MMC embryos cell numbers are reduced to about 14% of normal but that mitotic activity is much higher (Table 1). In a study of the time course of development following MMC injection at 7 d p.c. (zero time) dead cells (pycnotic granules) are observed at 1½ h and are most abundant at 6 h. Mitotic activity falls to its lowest at 6 h. The high mitotic activity in 7½-d MMC embryos seems therefore to reflect accelerated, compensatory growth in the embryo.

At 8½ d MMC embryos are still small and are now morphologically retarded (Fig. 1c, d). Headfolds are usually very small, or absent, the anterior end of the embryo usually being at the neural plate stage, that is, about 12 h retarded. In some embryos the posterior end seems more advanced, with a well developed allantois and occasionally with an early hind gut and one or two somites. The mitotic index is high throughout the embryo. Control embryos at this stage have raised head folds, neuromeres, up to seven pairs of somites, a beating heart, and fore and hind gut, and chorio-allantoic fusion is complete.

We initially thought that MMC embryos would die, but at 10½ d, although still small, they were almost normal. Head development showed no outward sign of retardation, but somite numbers were low (29 against 35 pairs in controls) and hind limb bud development was late in many. The course of development of MMC embryos is shown in Table 2. By 13½ d the only evidence for retardation in MMC embryos is that the mean fetal weight is significantly low (126 ± 3.0 mg, 5 litters, 42 embryos in MMC mice against 143 ± 3.4 mg, 4 litters, 42 embryos in controls,  $t = 3.1$   $P < 0.0025$ ). Abnormalities were found in one litter. Among the 10 embryos there were four cases of microphthalmia and one cleft face. There were no abnormalities in controls.

Litter sizes were a little lower in MMC-treated females, all embryo mortality occurring before 13½ d of gestation. Seventeen control litters averaged 10.9 implants and 3.2% mortality, whereas in 72 MMC litters these figures were 10.7 and 10.0%.

At birth MMC newborn animals were not noticeably small but were not weighed: 6% were stillborn. In 14 litters (mean size 8.0) microphthalmia was seen in 5, in 26% of all 14-d-old mice. No other abnormalities were seen. Growth was often slow and mortality was extremely high (25%, 53% and 64% being dead at 7 d, 14 d and 21 d, respectively). Of 14-d survivors, 50% were runted. Two entire 14-d litters had the appearance of 8-d-old mice, none had its eyes open. Only 30% (29 mice) were successfully weaned.

All runts and many of their larger littermates showed motor defects. Gait was slow and stumbling and many trembled violently. Most were reluctant to move and some were easily knocked over and took 5–10 s to get back on their feet. One extreme case would lie on its side for 30 s or more before moving.

Most runts and those with the worst motor defects died before weaning. Wilson razor blade slices were made of four severely affected 14-d mice but no internal abnormalities were found, all organs being present, correctly located, of normal proportions and with no noticeably enlarged cavities. Survivors generally seemed normal at weaning.

Fertility is low. All 29 survivors were test-mated to normal Q mice of proven fertility. All 18 males mated but 8 gave sterile plugs. Histological examination revealed small testes deficient in germ cells, many tubules containing only Sertoli cells. Of the 11 females 2 were sterile; both had small ovaries with about half as many follicles as controls, and one also developed motor defects after weaning. Histological examination of MMC embryos between 7½ and 11½ d in which alkaline phosphatase-positive primordial germ cells (PGC) were counted indicated severe depletion of PGCs in all MMC embryos up to 9½ d. Subsequently about half the embryos show good recovery of germ cell numbers. Migration of PGCs was retarded, even if allowance was made for the general developmental retardation.

Several other studies have used doses of MMC similar to or higher than those used here but none has started as early in pregnancy<sup>9–11</sup>. They cover the period 7½–15 d p.c.; all report high levels of induced abnormalities, up to 90% of females presenting abnormal fetuses and 64% of fetuses being affected. These figures agree well with the frequency of severe developmental retardation seen in our 8½-d embryos (100% of females present, up to 75% of fetuses affected), but do not correspond at all with the level of abnormality seen at late gestation. As all our experiments commence in pre-organogenesis stages this is perhaps not surprising. What our study does show is that the mouse embryo can withstand a major disturbance in its early development and recover to such an extent that structural abnormalities rarely emerge.

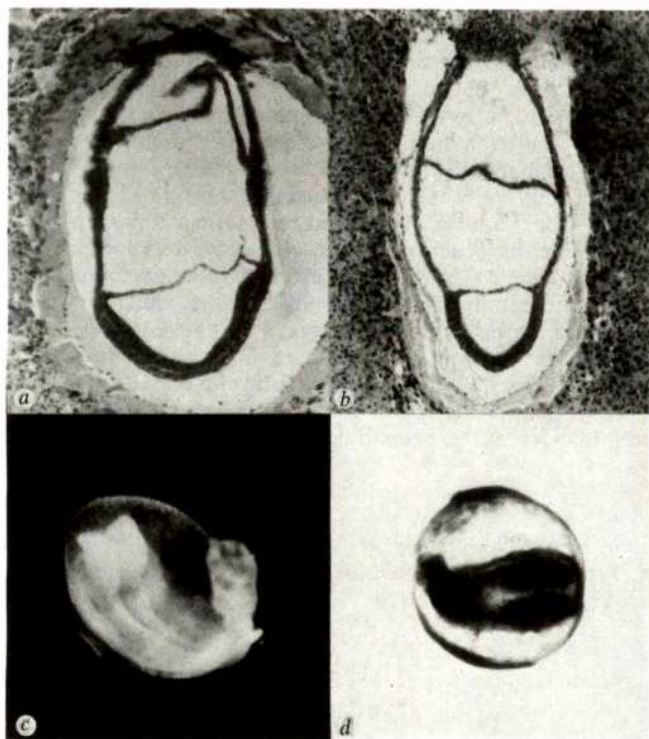
The frequency and nature of abnormality found in our 13½-d embryos gives little suggestion of the profound effects on post-

**Table 2** The course of development of embryos exposed to MMC at 6½ or 7 d postcoitum

Age at autopsy (d p.c.)	Age at injection	% of embryos showing:							
		No. of litters	No. of embryos		Headfold			Limb-bud	
			Live	Dead	Absent*	Normal	Somites	Fore	Hind
8½	—	6	56	2	0	100	91	0	0
	6½	6	46	5	44	56	56	0	0
	7	10	97	9	75	25	39	0	0
9½	—	5	58	3	0	100	97	73	0
	6½	5	44	2	0	100	86	43	0
	7	12	118	8	4	96	80	6	0
10½	—	2	23	1	0	100	100	100	100
	6½	6	56	17	0	100	95	85	80
	7	4	42	4	0	100	100	92	55

\* This figure includes embryos in which the neural plate has just started to crease, but raised neural folds are not observable.





**Fig. 1** *a*, Sagittal section through normal  $7\frac{1}{2}$ -d egg cylinder. *b*, As *a* for MMC-treated embryo. *c*, Normal  $8\frac{1}{2}$ -d embryo, dark ground illumination. *d*, MMC-treated  $8\frac{1}{2}$ -d embryo, bright field illumination. No headfold or somite development has occurred.

natal development and fertility, of MMC applied during pre-organogenesis stages. However, MMC-induced reduction in fertility has been reported to follow treatment given in mid-gestation<sup>8</sup>.

We have shown that the mouse embryo can be reduced to around 10% of its normal size at a time when it is about to begin organogenesis, but is nearly normal again before that phase of development is complete. Much of this regulation, especially morphogenesis, is accomplished within 48 h of the damage being inflicted. In particular, the formation of the brain and head seems to be achieved in about half the time it normally takes. During this period of accelerated development the embryo may be more susceptible to damage which would normally be trivial. It may explain, for instance, why some teratogens have their severest effect when administered as a series of small doses rather than as a single large dose<sup>5,12</sup>, a possibility not considered by Wilson in a discussion of the complications introduced by repeated dosing<sup>5</sup>.

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## Hybrid sterility in meadowlarks

THE eastern meadowlark (*Sturnella magna*) and western meadowlark (*S. neglecta*) are sibling species of songbirds in secondary contact throughout a narrow zone of sympatry in central North America<sup>1–3</sup>. Field studies suggest that specific differences in vocalisations are the principal isolating mechanism and that hybridisation is limited in extent and possibly restricted to regions of recent contact<sup>4–7</sup>. The incidence of hybridisation is difficult to determine, however, due to their similar appearance, and there is uncertainty as to the recognition of some hybrids of known parentage<sup>7</sup>. Multivariate analysis alone offers no additional resolution to the problem<sup>8</sup>. A programme to breed and hybridise meadowlarks in captivity is one approach to determining the viability and fertility of hybrids. The difficulties inherent in the technique and the time required to amass adequate samples have precluded its use among avian systematists, with the exception of those working on some game species<sup>9,10</sup>. However, I now report that between 1966 and 1978 I was successful in inducing 25 captive meadowlarks to pair and produce 44 clutches of 158 eggs. Mixed matings among non-hybrids resulted in 90% fertility, not significantly different from the 87% fertility among eggs from pure matings, whereas the fertility of eggs from pairing of hybrids was only 10%. All eggs resulting from pairing the one surviving backcross hybrid were infertile.

Indicative of the infertility of my hybrid meadowlarks is the performance of three siblings produced in my aviary in 1975, from a mixed pairing of a male *S. magna* and a female *S. neglecta*. The three hybrids, two males and a female, did not breed at 1 yr of age, although paired with experienced mates. In 1977 the female hybrid was paired with a male *S. neglecta* that had bred successfully the previous year. She built a typical meadowlark nest, laid a clutch of four eggs, and incubated with normal attentiveness. On the eighth day of incubation I determined that all eggs were infertile. The male *S. neglecta* demonstrated his continued fertility by breeding successfully the following year. One of the male hybrids was paired in 1977 with a female *S. neglecta* that had bred successfully 2 yr earlier. She laid a clutch of four eggs determined to be infertile after 6 days of normal incubation. Also in 1977, the second male hybrid was paired with a 2-yr-old female *S. magna*, who laid a clutch of three eggs that proved to be infertile after 10 days of normal incubation. This female laid fertile eggs the following year in a mixed pairing with a male *S. neglecta*.

My most extensive and convincing data were accumulated on a male hybrid produced from a mixed pairing of a male *S. neglecta* and a female *S. magna* in 1966 (the first meadowlark ever produced in an aviary and raised to maturity). At 1 yr of age he was backcrossed with his mother, who laid a clutch of four eggs that proved to be infertile after 11 days of normal incubation. At 2 yr, he was backcrossed again with his mother, who laid three clutches and a total of seven infertile eggs. During that same season he was paired with two different hybrid females. These crosses of F<sub>1</sub>'s resulted in three clutches and a total of 12 infertile eggs. In 1971, at 5 yr of age, he was paired with a female *S. neglecta*, a proven breeder who laid fertile eggs in a mixed pairing that same year and continued to produce fertile eggs over the two ensuing years. This backcross resulted in a clutch of four infertile eggs incubated normally for at least 8 days.

The only fertile eggs from a pairing of a hybrid were laid by a female hybrid produced in the wild near Poughkeepsie, New York, from a mixed pairing between a male *S. neglecta* and a female *S. magna*<sup>7</sup>. This female hybrid was first successfully paired in 1968, when pairings with a male hybrid produced two clutches and a total of eight infertile eggs. In 1971, at 9 yr of age, she produced two fertile clutches: one of four eggs in a pairing with a male *S. neglecta*, and one of three eggs in a pairing with a male *S. magna*. However, in that same year she laid four infertile



eggs in a pairing with another male *S. neglecta* who had already bred successfully earlier in the season, and she laid a clutch of eggs in a second pairing with the male *S. magna* cited above, but the three eggs in this subsequent attempt proved to be infertile. A sibling female hybrid from the mixed pairing near Poughkeepsie was paired with one of my aviary-produced hybrids in 1968 and produced four infertile eggs. I was unable to establish breeding pairs with the other hybrids from Poughkeepsie.

Two of the backcross hybrids by the female hybrid cited above were hand-reared, but only one of these (a male, from W × F<sub>1</sub>WE) was subsequently involved in a successful pairing. When 2 yr of age this backcross hybrid male was paired first with a female *S. neglecta*, resulting in five infertile eggs, and later with a female *S. magna*, resulting in three infertile eggs. The female *S. neglecta* had bred successfully in my aviaries during each of the previous two years; the female *S. magna* produced fertile eggs before and after this unsuccessful pairing with the backcross hybrid.

The ease with which hybrids were produced from mixed pairings of captive birds, given no choice of mates, tells us little about the incidence of hybridisation of meadowlarks in the wild, where behavioural and ecological isolating mechanisms effectively limit mixed pairings throughout most of the zone of

sympatry. The sterility of the captive hybrids is significant, however, and suggests that when infrequent hybridisation does occur in natural populations, the hybrids are at a disadvantage and are selected against, a conclusion that had been suggested earlier by Rohwer's multivariate analysis of specimens from a region of sympatry<sup>8</sup>. The causal factors for the sterility are as yet unknown, but apply to hybrids of both sexes and to hybrids resulting from both categories of mixed pairings. Efforts to find abnormalities in the chromosomes of the hybrids have thus far been unsuccessful. The reduction in fertility is not accompanied by an obvious reduction in viability, for the captive hybrids appeared to be as healthy and vigorous as other captives.

I thank the following for assistance in maintaining the captive birds: Mrs Vicky Lanyon, the late Edward Szalay (who was superintendent of the Kalbfleisch Field Research Station) and James Mansky (current superintendent of the station).

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**Table 1** Fertility of eggs produced by captive eastern (E) and western (W) meadowlarks

Category of pairing	No. of fertile/infertile eggs in complete clutches	Total no. of fertile/infertile eggs	% Fertility
<b>Pure pairings</b>			
W	4/0, 3/0, 3/0, 2/0, 2/2	17/2	0.89
E	3/1	3/1	0.75
All pure pairings		20/3	0.87
<b>Mixed pairings (male × female)</b>			
E × W	5/0, 5/0, 4/0, 4/0, 4/0, 4/0, 3/0, 2/0, 2/0	33/0	1.00
W × E	4/0, 4/0, 4/0, 4/1, 3/0, 3/1, 0/4*	22/6	0.79
All mixed pairings		55/6	0.90
<b>Pairings of hybrids (male × female)</b>			
F <sub>1</sub> WE × E	0/4, 0/3, 0/2, 0/2	0/11	0
F <sub>1</sub> WE × W	0/4	0/4	0
F <sub>1</sub> EW × E	0/3	0/3	0
F <sub>1</sub> EW × W	0/4	0/4	0
W × F <sub>1</sub> WE	0/4, 4/0†	4/4	0.50
W × F <sub>1</sub> EW	0/4, 0/4, 0/3	0/11	0
E × F <sub>1</sub> WE	0/3, 3/0†	3/3	0.50
E × F <sub>1</sub> EW	0/4, 0/3	0/7	0
F <sub>1</sub> WE × F <sub>1</sub> WE	0/4, 0/4, 0/4	0/12	0
Backcross hybrid × W	0/5	0/5	0
Backcross hybrid × E	0/3	0/3	0
All pairings of hybrids		7/67	0.10

Earlier attempts to breed meadowlarks failed, presumably because the flight pens were too small and offered an insufficient area of natural sod for nesting substrate<sup>7</sup>. Ultimately I resorted to aviaries located in a grassland habitat at the American Museum of Natural History's Kalbfleisch Field Research Station at Huntington, New York, that measured 12.2 × 4.9 × 2.7 m, thus providing 60 m<sup>2</sup> of sod per breeding pair and ample unobstructed space to permit the aerial components of courtship. The sources of the breeding stock were as follows: seven *S. magna* captured in New York and New Jersey (three as adults and four as nestlings); five *S. neglecta* captured as adults in California; four *S. neglecta* produced in my aviaries from pairings of the California birds; one *S. neglecta* trapped as a breeding adult near Poughkeepsie, New York<sup>7</sup>; six hybrids produced in captivity from the foregoing stock, and two hybrids produced naturally near Poughkeepsie, New York<sup>7</sup>. Mixed pairings were as readily achieved in the flight pens as were pure pairings. No choice experiments were conducted. Whenever possible hybrids were paired with mates known to have bred successfully to maximise the significance of the performance of the hybrids. Fertility of eggs was established by candling after a minimum of four days of incubation, when the embryos and air sacs are clearly visible.

\* This clutch involved a male in at least its tenth year of age (1978) that bred successfully between 1970 and 1975; he was not paired with hybrids after 1975.

† These eggs were laid by the same female F<sub>1</sub> hybrid and were the only fertile eggs produced by a hybrid; four other clutches (15 eggs) produced by this hybrid were infertile.

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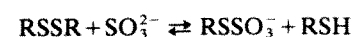
## Errata

In the letter 'Common regulatory mechanism of expression and conjugative ability of a tetracycline resistance plasmid in *Bacteroides fragilis*' by Privitera *et al.*, *Nature* **278**, 657, in paragraph 2 line 11 the molecular weights of the plasmids should read 27 and 28 × 10<sup>6</sup> and in line 15, 17 × 10<sup>6</sup>. Similarly, in paragraph 11, line 3 the molecular weight should read 17 × 10<sup>6</sup>.

In the letter by H. J. Chapman, *Nature* **277**, 642, the word 'age' was omitted from the title which should read '2,390 Myr Rb–Sr whole rock age for the Scourie dykes of north-west Scotland'.

## Corrigenda

In the letter 'Sulphonation of cholinergic receptor disulphide bond increases response to ACh' by A. Steinaker, *Nature* **278**, 358, lines 9–10 and the equation should read: The active agent is the sulphite ion, the reaction being



In the review article 'Facts and hypotheses of molecular chemical tunnelling' by V. I. Goldanskii, *Nature* **279**, 109, on page 113 in the left-hand column line 45, for 'Sagan<sup>73,76</sup>', read 'Sagan<sup>73</sup> and Sagan and Khare<sup>76</sup>'. In line 58 for 'Sagan considers' read 'Sagan and Khare consider' and in line 71 for 'According to Sagan<sup>73,76</sup>' read 'According to Sagan<sup>73</sup> and Sagan and Khare<sup>76</sup>'.

# reviews

## Arena of technological policy

Dorothy Nelkin

*Directing Technology.* Edited by R. Johnston and P. Gummert. Pp. 271. (Croom Helm: London, 1979.) £10.95.

BELIEF in the efficacy of technological progress is increasingly tempered by awareness of its ironies. Technological 'improvements' may cause disastrous environmental problems: drugs to stimulate the growth of beef cattle may cause cancer; 'efficient' industrial processes may threaten the health of workers; beneficial biomedical research may be done at the expense of human subjects; and a new airport may turn a neighbourhood into a sonic garbage dump. Even efforts to control technology may impose inequities, as new standards and regulations pit the quality of life against economic growth and the expectation of progress and prosperity. Indeed, the public policy discourse on directing technology has changed remarkably in the past ten years. How to promote and utilise technology for socially useful ends remains an important but no longer central issue; attention has shifted to a greater emphasis on ways of assessing and controlling potentially undesirable impacts.

*Directing Technology* is a book of thirteen essays edited by Ron Johnston and Philip Gummert, both from the Department of Liberal Studies in Science at the University of Manchester. The essays address the two thrusts of government involvement in the direction of technology: promotion through the encouragement of research and development, and control through regulation. Government intervention has increased in recent years, fostered by the growth and the scale of public investment in technology, by the importance of technology in international economics and politics, and by the increase in public criticism and concern. Various patterns of government promotion of technology through encouraging research and development are explored in six case studies in the

first part of the volume. They include cases in Western Europe (Keith Pavitt), Canada (Ian Chapman) and Great Britain (Philip Gummert). Examples are drawn from specific industries: nuclear power (Roger Williams), aviation (John Hartland and Michael Gibbon), and motor vehicles (Peter Stubbs).

The second half of the book focuses on mechanisms of control. The focus is primarily on the problem of safety, with chapters on the regulation of the automobile industry (Judith Reppy), on the environmental impacts of technology (Dave Ewa and Harry Rothman), on pesticides (Brendan Gillespie), and on recombinant DNA (Edward Yoxen). Various mechanisms of control are discussed in this section: technology assessment (Michael Gibbons) and the role of parliaments (John Hartland).

The book concludes with a most interesting article by Geoffrey Price, which—perhaps deliberately—serves as a critique of the entire volume. Price observes that policies for promotion and control of technology express social goals and priorities:

The differing approaches to the control of technology are themselves an expression of the basic assumptions made within different political traditions about the coordination of general social goals with the detailed activities needed to attain them. In short, approaches to the regulation of technology are themselves particular instances of the political assumptions which articulate a given society—whether they be those of centralised planning, Burkean conservative liberalism, or radical communitarianism.

Price goes on to argue, as I would, for more overt recognition of the political context of decision-making concerning the direction of technology. The political assumptions underlying technological decisions are often unstated, because it is convenient to maintain an image of neutral technology. But clearly decisions about promotion and control have significant social and political consequences. They may effect employment, the distribution of income,

or occupational health and safety. The collaboration between industry and government discussed throughout this volume may have serious political implications for the ability to control technology, as the functions of promotion and control necessarily come into conflict. Can we talk these days of directing technology without discussing the problems of scientific research as well? Can we talk about the government's role in the promotion of technology without dealing with the contradiction between its efforts to promote as well as to control?

Although this book is a very useful and timely collection of articles, I wish the editors had presented a detailed and critical analysis of such politically controversial problems. Several of the authors touch on political issues; the declining capacity of parliament to influence technology, or the secrecy of government institutions in dealing with the problem of pesticides. But, for the most part, the articles are relatively bloodless and bland. It is as if decisions in this highly political arena of technology policy—political in the sense of its consequences for the distribution of costs and benefits—occur in an ideological vacuum.

One way to approach these political assumptions would be to develop the comparisons implicit in the essays. Different countries approach common technological problems in different ways depending on political expectations and cultural styles. Comparative analysis can reveal assumptions underlying decision-making processes and they can suggest a wider range of policy options. A lack of comparative analysis more generally marks the field of science and technology policy. This book, with its case-studies from different countries, at least provides us with the opportunity. □

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## Speculations on micromolecular evolution

*The Genetic Mechanism and the Origin of Life.* By L. S. Dillon. Pp. 563. (Plenum: New York and London, 1978.)

I FOUND this to be a strange book. The list of almost 3,100 references occupies 127 of its 562 pages. This does not include the space in the text occupied by citations; I counted 23 on one page. Obviously, only perfunctory mention can be made of most articles to which the citations refer. An analogy to describe the organisation of the first five chapters is that it seemed as if the author had been out in the fields with a butterfly net, industriously scooping up insects, which he sets before us in a display bewildering in its variety. His collection of topics includes everything from the origin of life, to the complete two-dimensional structure of MS-2 RNA, and the amino acid sequences of six calf-thymus and sea-urchin histones.

After this *tour-de-force*, the author proceeds to invent new theories from the stepwise evolutionary formulation of larger amino acids from smaller ones, such as alanine from glycine, and valine from aspartic acid. He says, for example, "In the author's laboratory, continuous bubbling of CO<sub>2</sub> through a 0.1 [sic] aqueous solution of alanine at ambient and elevated temperatures for a week failed to produce any detectable aspartic acid (Dillon, unpublished)", but he doesn't give up; he next says, "Hence, the addition can probably be made only through enzymatic mediation". He does not mention that aspartic acid is formed biologically by transamination, but not from alanine. Grandiose but unrealistic schemes are also set forth for "chemical evolution" of lysine, isoleucine and other amino acids from aspartic acid.

In chapter 7, Dillon proceeds to take sequences of tRNAs apart into small pieces and put them together again. He says that the absence of unmodified adenosine from the first site in anticodons is included in constraints which "to the author's knowledge . . . have not been previously noted in the literature". Actually, this is a well-known characteristic and has a familiar explanation, not mentioned by Dillon. Adenosine in the first position of anticodons is deaminated to inosine, (*Biochim. Biophys. Acta*, **213**, 352, 1970), which wobble-pairs with three different bases. The incorrect statement is made on p296 that "the triplet -CAA is consistently present at the 3'-terminus of the molecule" of tRNA.

The next chapter, on "tRNA evolution", attempts at great lengths to construct a phylogeny by comparing base sequences of various arms of tRNA molecules. Such a procedure is at variance with results obtained by aligning and comparing the complete sequences of tRNA molecules (*J. Molec. Biol.* **78**, 91; 1973), a method which shows that sequence changes have been so extensive as to obscure any phylogenetic relationship of nearly all tRNAs except for a few closely related pairs.

In chapter 9, Dillon goes butterfly-collecting for odds and ends among the structures of viruses. At one point he briefly notes, but does not describe, the "blocked complex 5'-terminal struc-

ture" in reovirus and polyhedrosis virus RNA, discovered by Miura *et al.* (p402). This is, of course, the "cap" of eukaryotic mRNA, an appendage which Dillon (p131) strangely ignored in describing the structure of eukaryotic mRNA. The book concludes (chapter 10) with highly imaginative (or imaginary) and grandiose scenarios for the origin of life, including schemes for the stepwise evolution of small viruses into large ones. The voluminous bibliography, following chapter 10, is probably the most useful feature of the book.

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## Protein phosphorylation

*Cyclic Nucleotides, Phosphorylated Proteins, and Neuronal Function.* Distinguished Lecture Series of the Society of General Physiologists. Vol. 1. By P. Greengard. Pp. 124. (Raven: New York, 1978.) \$15.60.

IN this slim volume, Professor Greengard expounds his theory that protein phosphorylation is the final common pathway that mediates the biological effects of a wide variety of regulatory agents in addition to those that are known to act through cyclic AMP. It is really an extension of the 'second messenger' theory of cyclic AMP function, in that protein phosphorylation is depicted as the immediate and physiologically important result of increased cyclic AMP levels. However, Professor Greengard is nothing if not catholic in his enthusiasm for protein phosphorylation, and the actions of steroid and peptide hormones, insulin, interferon, all the neurotransmitters (excepting acetylcholine at nicotinic synapses) as well as the diverse effects of calcium ions are all envisaged by him as proceeding by this mechanism.

One's unease develops as the list increases; can the expression of all this biological diversity really be channelled through the simple phosphorylation of protein? It is clear that the regulation of glycolysis proceeds by cyclic AMP-induced protein phosphorylation. Other cases exist, but the argument from the particular to the general is not well founded. The mere correlation of varied cyclic nucleotides with protein phosphorylation in various circumstances is necessary but not sufficient to demonstrate it. It may be that protein phosphorylation is a side-effect of increased cyclic nucleotide levels rather than a mandatory stage in the expression of

their regulatory activity.

And even if protein phosphorylation was a mandatory stage, does this tell us much about the processes anyway? After all even if the theory were true, the important questions surely are; what proteins in each individual case are phosphorylated and how does this affect their physiological activity? Professor Greengard would probably agree that these questions are important and protest that he is merely proposing a working hypothesis for the detailed investigation of individual cases. But the trouble is that protein phosphorylation as a working hypothesis is so general and vague that it does not really help us formulate specific questions about regulatory mechanisms.

I turn now to the particular role of protein phosphorylation in neuronal and synaptic activity, Professor Greengard's special interest. He has proposed a scheme whereby a neurotransmitter (presumably the author has noradrenaline in mind, though it is not specified) is bound to its postsynaptic receptor and activates adenyl cyclase; this stimulates protein phosphorylation which causes a permeability change and thus a change in the membrane potential. Now, as Professor Greengard shows, the phosphorylative and enzymic activities prescribed by the scheme are found in the synaptic region but it is by no means clear that phosphorylation of the membrane protein causes the membrane conductance change that is known to be caused by transmitter action. The best experimental evidence that the author can offer is that isoproterenol (known to cause an increase in cyclic AMP) causes in turkey erythrocytes an increase in sodium influx, and protein phosphorylation, the dose- and time-response curves of which are similar. There are various correlations but none distinguishes between protein phosphorylation being a result of an increased ion flux rather



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A translation from the Polish of a manuscript smuggled out of the Warsaw Ghetto that details studies performed by the resident Jewish physicians on patients suffering from Nazi-imposed starvation. Some of the findings were firsts in the medical literature and others remain unique today. Each chapter of the book is followed by extensive comments by the editor — bringing the information up-to-date and outlining the most recent findings in the particular area discussed. Topics include: clinical changes in adults; clinical changes in children; metabolic adaptations including carbohydrate and acid base metabolism; pressure, venous and arterial pressure, oxygen consumption, and cardiac work measurements; changes in the eye and in vision; changes in the blood and bone marrow; and pathologic anatomy. (Series: *Current Concepts in Nutrition Vol. 7*)

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than its cause. I believe this to be crucial; but even if it were conceded it is a far cry from the permeability changes of the turkey erythrocyte membrane, measured in minutes, to that of the synapse measured in milliseconds. Indeed, the whole problem of timing is ignored by Professor Greengard. As it is proposed that the membrane conductance change results from the sequential action of two enzymes, it is surely incumbent on the author to show that the substrate concentration and enzyme activities are sufficient to enable protein phosphorylation to be fast enough to account for the speed of the permeability changes.

In another section Professor Greengard described his experiments in which calcium influx into synaptosomes causes a phosphorylation of a particular protein (protein I) that he has identi-

fied as being only present in the synaptic region. This is interesting, but he concludes (p77) "that calcium is a regulatory agent some of whose effects may be mediated by protein phosphorylation". It may; but these experiments have only demonstrated that protein phosphorylation is one of the many sequelae of depolarisation in the presence of calcium, which is not the same thing at all. And it is this type of reasoning which eventually alienates support from the author's general hypothesis; this is a pity, as the subject of protein phosphorylation, to which Professor Greengard has made distinguished contributions, deserves careful analysis without preconception as to its rôle.

**R. M. Marchbanks**

*R. M. Marchbanks is Senior Lecturer in the Department of Biochemistry at the Institute of Psychiatry, London, UK.*

## Photosynthetic bacteria

*The Photosynthetic Bacteria.* Edited by R. K. Clayton and W. R. Sistrom. Pp. 946. (Plenum: New York and London, 1979.)

THIS very ambitious book attempts to provide an overall review of the present state of knowledge on all aspects of the study of photosynthetic bacteria at the highest level. It contains review articles by experts in many fields reflecting the importance of photosynthetic bacteria as excellent "simple" systems for studies of photosynthesis, membrane biogenesis, heme and carotenoid biosynthesis, and so on. This encyclopaedic approach has advantages and disadvantages. The main advantage of this book is that it provides an enormous amount of basic information about photosynthetic bacteria in readily accessible form. It must therefore become an essential reference work for any laboratory doing research on these organisms. However, the book has a number of disadvantages which arise from the attempt to be completely comprehensive. Almost every topic is covered by two chapters by different authors. This results in considerable duplication of material, even to the extent of printing the same electron micrograph in two chapters, and of course in conflicting interpretations. The result is to make the book much larger and more difficult to use than is required by the subject.

A more serious problem is that the book is out of date. It would seem that many, although not all, of the

chapters were completed in 1975, with minor updating to 1977. Although this is not too important for much of the background information, it is disastrous for articles dealing with rapidly moving research areas. The problem is made worse by variations in the amount of updating authors have done, and the confusion of multiple chapters dealing with each subject. For example, the chapter on primary electron acceptors does not mention the role of bacteriopheophytin in the reaction centre, because, as the author states, it was written in 1975 before this work was published. The work is in fact described in varying detail in three other chapters, although the chapter titles would not lead the uninitiated reader to look in them for this information.

The book is an excellent source of information on photosynthetic bacteria, but it must be used with a fairly critical attitude. It cannot therefore be recommended to non-specialists as a completely satisfactory dictionary of its subject.

**M. C. W. Evans**

*M. C. W. Evans is Reader in Plant Chemistry at University College, London, UK.*

● An English language edition of *Einstein* by Louis de Broglie, Louis Armand, Pierre-Henri Simon *et al.*, originally published in French by Hachette (Paris) in 1966, has now been published by Peebles Press International (New York) and David and Charles (Newton Abbot, UK) at £7.50.

● *The Runaway Universe* by Paul Davies (for review, see *Nature*, 272, 785; 1978) has been published in paperback as *Stardoom* (Fontana: London), price 95p.

## Capture-recapture methods

*Investigating Animal Abundance.* By M. Begon. Pp. 97. (Edward Arnold: London, 1979.) Paperback £3.95.

STUDYING the population dynamics or genetics of a natural population requires some knowledge of the changes in absolute population density over a period of time or between different locations. From this follows the opportunity to quantify the processes of birth and death, emigration and immigration. Adequate techniques for estimating population size are therefore of crucial importance to the study of natural populations.

Michael Begon has gathered together in a short volume a guide to a family of techniques for estimating absolute population size, all involving the mark, release and recapture of individuals from a population. These techniques (for example, Lincoln Index, Bailey's Triple Catch, Fisher-Ford Method, Jolly's Stochastic Method and Manly-Parr Method) have proved useful in the study of many populations from fruit flies, mosquitoes and grasshoppers to brown rats and salmonids. In the past, these capture-recapture methods have been a favourite hunting ground for statisticians who have delighted in the underlying theory. Begon is a biologist and has written for biologists, hoping to show "the essential simplicity of the mathematical techniques" and their potential as a biological weapon. In this he succeeds by virtue of a succinct prose, a clear lay-out and the good use of worked examples.

Capture-recapture methods are, however, only one of many techniques for estimating population size. This is very clear from T. R. E. Southwood's book, *Ecological Methods* (Chapman and Hall: London, second edition; hardback £10, paperback £3.75; for review, see *Nature* 278, 673; 1979), where over 50 pages are devoted to mark-release-recapture, but also nearly 100 pages to other methods for estimating absolute population size and a further 50 or so to techniques for relative population estimates. With Begon's aim of putting the biologist and his problems foremost, I would have liked to see, if only in passing, the subject viewed in its wider context.

Nevertheless, this book will be valuable for students who have to learn capture-recapture methods, for their teachers or for anyone contemplating their use in a research project.

**M. P. Hassell**

*M. P. Hassell is Reader in Insect Ecology at Imperial College, University of London, UK.*



## One-dimensional conductors

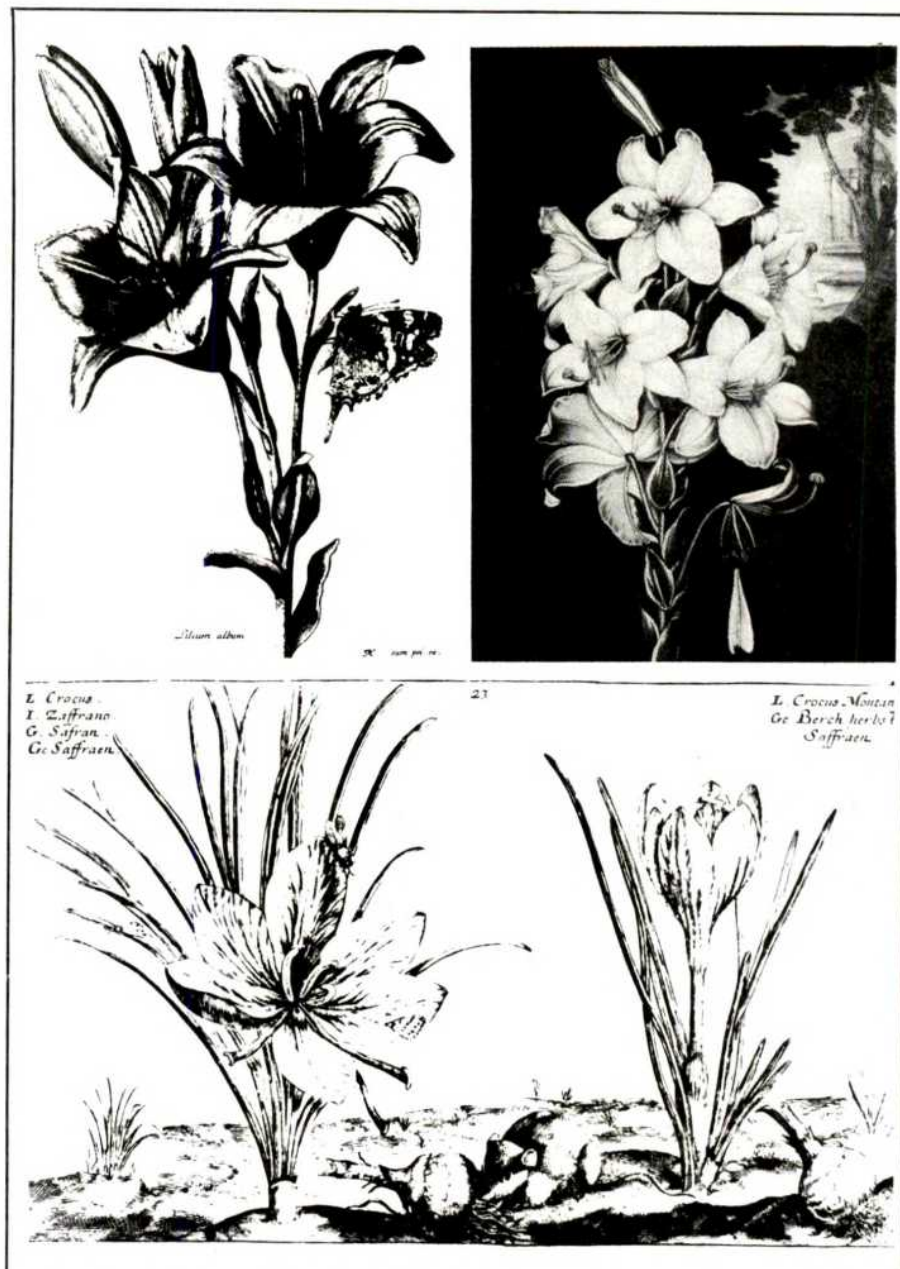
*Highly Conducting One-Dimensional Solids.* Edited by J. T. Devreese, R. P. Evrard and V. E. Van Doren. (Plenum: New York and London, 1979.) £26.77.

'ONE-DIMENSIONAL CONDUCTORS' are very popular at present because of the great diversity of physical phenomena which they exhibit. There are, of course, no truly one-dimensional conductors, and what we are dealing with are highly anisotropic solids composed on linear or chain-like structures. The systems which are discussed in some detail in this book are KCP or  $K_2Pt(CN)_4 \cdot Br_{0.3} \cdot xH_2O$ , and the organic charge transfer system TTF-TCNQ and its derivatives. In these solids the electronic anisotropy is the result of the directional nature of the orbitals involved in the formation of the conduction band. Mechanically, these two systems are almost isotropic. Other very anisotropic systems would include the  $(SN)_x$  polymer,  $NbSe_3$ , and polyacetylene-halogen complexes.

One of the main interests in the study of these systems is the search for high electrical conductivity or high temperature superconductivity. A theoretical paper by Little in 1964, in which an electron-exciton interaction was proposed as a likely mechanism for high temperature superconductivity has been an underlying influence in this work. Following this, initial reports by the Pennsylvania group in 1973 on the high conductivity of TTF-TCNQ below room temperature, sparked off the present spate of activity. Since then, the observation of incommensurate superstructures, which have been interpreted as Peierls distortions, forms an important aspect of this work. The degree to which one dimensional fluctuations depress the Peierls transition to zero temperature, and the competition between electron-electron coulomb repulsion and electron-phonon coupling on the Peierls transition, provide some of the main interests which are discussed in great detail in this book.

This is the most coherent treatment which has so far been given on the subject. The book consists of eight chapters written by well known experts deeply involved in the subject. The chapters are by Berlinsky, Comes and Shirane, Heeger, Schultz and Craven, Sham, Emery, Gutfreund and Little, and Bardeen.

It is, of course, impossible to cover even the main points in a short review. The book leans heavily on the more physical aspects, and even here perhaps on the more theoretical problems. This



Top left, Madonna Lily, etching by Nicolas Robert, from his *Diverses Fleurs* (1675); top right, White Lily, aquatint by J. C. Stadler, in R. J. Thornton's *Temple of Flora* (1799-1807); bottom, Crocuses, line engraving by Crispin de Passe, from his *Hortus Floridus* (1614).

A new exhibition, entitled *Flowers in Art from East and West*, opened at the British Museum on 10 May and will continue until 9 September, 1979. The exhibition brings together a wide range of floral art, including the work of many of the greatest painters and draughtsmen working in many parts of Europe and Asia from the ninth century AD to the present day. The various techniques of European print-

making and the woodblock printing of the Far East are also illustrated. A book of the same title, from which the above illustrations are taken, is being published simultaneously by the British Museum Publications Department. Paul Hulton, Deputy Keeper of Prints and Drawings, and Lawrence Smith, Keeper of Oriental Antiquities, provide not an accompanying catalogue to the exhibition but a proper book which can be read on its own and which covers the development of floral art, starting with the early manuscripts of the Byzantine Empire. The book is beautifully produced, with both colour and black and white illustrations, in hardback (£7.95) and paperback (£3.95).

should not frighten off those interested in the more experimental or chemical side of this topic, as there is a good deal to learn from the contents. It is certainly the best treatment of the subject of one-dimensional systems that

I have seen, and it can be warmly recommended.

A. D. Yoffe

A. D. Yoffe is Reader in Physics at the Cavendish Laboratory, University of Cambridge, UK.



# obituary

## Leland J. Haworth

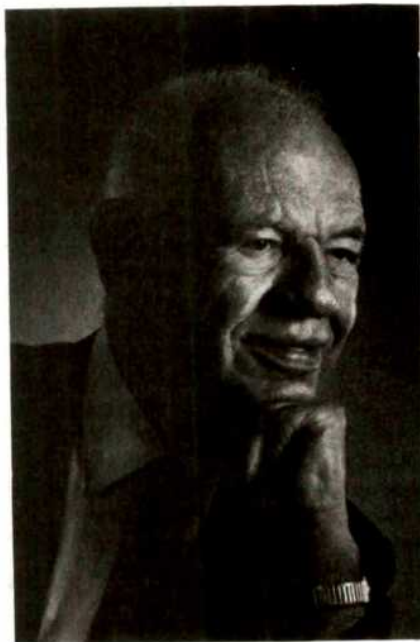
DR LELAND J. HAWORTH, prominent physicist, administrator and government official, died on 5 March 1979, in Port Jefferson, New York, at the age of 74. A former director of the Brookhaven National Laboratory, commissioner of the United States Atomic Energy Commission, and director of the United States National Science Foundation, he brought to those organisations a wealth of experience as a teacher, a research scientist, a project leader and a science administrator.

His activities were not restricted to science development in the U.S. only. A strong advocate of international co-operation in science, he played an active role in fostering such co-operation.

Leland Haworth first became involved with 'big science' when he was engaged in major microwave radar research and development programmes at the Massachusetts Institute of Technology's Radiation Laboratory from 1941 to 1946. His responsibilities grew from those of a components development engineer to those of head of the Receiver Components Division and a member of the laboratory's steering committee. His specialty was electronics, especially in data presentation and precision instrumentation.

Following a brief return to the University of Illinois as professor of physics, Haworth in 1947 joined the Brookhaven National Laboratory (BNL) as assistant director in charge of special projects. Brookhaven was established in 1946 by the United States Government and Associated Universities, Inc. (AUI) as a multidisciplinary research institution, a primary purpose of which was to provide special facilities essential for basic research in nuclear and related sciences.

Haworth was appointed director of Brookhaven in 1948. He stimulated the development of a truly user-oriented philosophy of service to the scientific community, including the development and provision of ever more advanced facilities. In the early 1950s the Cosmotron (a 3.0 GeV proton accelerator) and the graphite research reactor were brought into service. The 30 GeV alternating gradient synchrotron (AGS) was authorised in 1954, brought into operation in 1960, and today remains one of the key elements in the United States high energy physics programme. Similarly, under his



Brookhaven National Laboratory

direction, construction of the high flux beam reactor (40 MW) was initiated in 1959, and today it is still the major U.S. research reactor providing external high intensity beams of neutrons for research in physics, chemistry and biology.

Haworth's position as director of Brookhaven and his personal interest in high energy accelerator development were of great benefit to CERN both in the initial planning and development of that institution and in bringing the alternating gradient focusing concept, through CERN and BNL joint studies, to a reality in the Brookhaven AGS and the CERN proton synchrotron. This close cooperation between the two institutions has continued.

From 1951 to 1960, Haworth also served as vice president of Associated Universities, Inc. He was named president in 1960 and held that post, while continuing as laboratory director, until the spring of 1961.

Leland Haworth immersed himself completely in every activity he undertook. His drive, his insistence on facts and accuracy, his pursuit of excellence and his clarity of expression were hallmarks. If Haworth did it, it was done well. It might have taken longer but it was done correctly. His leadership and, above all, his consideration for staff and regard for their capabilities and achievements earned the respect and admiration of all of his associates.

Haworth's influence extended beyond Brookhaven: his services were in

demand as a member of numerous study groups and advisory panels—for the White House, the Department of Defense and other U.S. Government Agencies.

In 1961, President John F. Kennedy appointed Haworth commissioner of the U.S. Atomic Energy Commission. He rapidly became involved in matters of national security, civilian energy development and the support of fundamental research. As a commissioner, he was heavily involved in the nuclear weapons development programme and in the preparation of the 1962 AEC Report to the President on civilian nuclear power.

Haworth recognised the urgent need for restraint in nuclear weapons development, especially in the test programmes then carried out primarily in the atmosphere. He advocated a test ban that could be readily monitored by national means, an approach that led to the limited test ban treaty prohibiting testing in the atmosphere and under water.

The Atomic Energy Commission's 1962 Report to the President on civilian nuclear power was a masterpiece of its time. It was a major governmental energy planning document and, though its thrust was nuclear, reference was made to the need for conservation and to the development of coal resources. Haworth's analytical approach, rigorous logical presentations and other contributions to the study gave it substance and credibility and set the tone for what is now a necessary ingredient of all energy planning.

President Kennedy asked Haworth to leave the Atomic Energy Commission in order to become director of the National Science Foundation; he assumed office on 1 July, 1963. During Haworth's six-year term, he brought the foundation to maturity in a period that saw great expansion of federal support for the conduct of basic science. He guided the development of a programme which increased the number of high-quality institutions across the nation, and he pioneered NSF efforts to link fundamental science to applied science and technology.

Haworth returned to AUI in 1969 as special assistant to the president. He continued as a consultant to the president and to the BNL director following his retirement in 1977.

Leland John Haworth was born in Michigan on 11 July 1904. He grew

up in Indiana and attended Indiana University, receiving his AB in 1925 and AM in 1926, both in physics. In addition to his academic pursuits, he participated in varsity baseball and tennis. He received his PhD in physics from the University of Wisconsin in 1931.

He was an instructor in physics at the University of Wisconsin from 1930 to 1937. During his graduate work and until 1934, he did research in solid state physics, specialising in the surface structure of metals. In 1934 he switched to nuclear physics, which thereafter remained his principal field.

Following one year as a Lalor Fellow in physical chemistry at the Massachusetts Institute of Technology, Haworth, in 1938, joined the physics department of the University of Illinois. His association with Illinois continued until 1947 except for five years of war-time leave at MIT.

Dr Haworth was a member of the board of directors of Oak Ridge Associated Universities (ORAU) from 1959 to 1961, and again from 1971 to February 1978, when he was elected director emeritus—the first time ORAU had made such an appointment.

He was a member of the National Academy of Sciences, the American Philosophical Society, Phi Beta Kappa and Sigma Xi. He was a fellow of the American Physical Society, the New York Academy of Sciences, the American Academy of Arts and Sciences, and the American Nuclear Society (member of board of directors, 1955–60; president, 1957–58).

For his work during World War II, Haworth received the President's Certificate of Merit. A mesa in the Antarctic and an asteroid have been named in his honour. He was the recipient of many honorary degrees.

The name of Haworth is synonymous with meticulous care for the work at hand. His traits of objectivity, attention to detail and sensitivity to the problems of others influenced all who worked with him, and we are the better for it. The institutions with which he worked have been left with a Haworth heritage, and they are the better for it.

Gerald F. Tape

## W. R. Aykroyd

DR WALLACE RUDELL AYKROYD, CBE, who died on 7 February 1979, aged 79, was a nutritionist of international renown since the early days of his career. His ability for meticulous work and great clarity of thought were soon recognised and his powers of organisation and administration left their mark in the many posts of distinction he held, particularly as the first Director of the Nutrition Division of the Food and Agriculture Organisation of the United

Nations from 1946 until 1960. His capacity to marshal facts and produce scientific reports in stylish and fluent prose made his publications important and influential in the fields of nutrition, public health and agriculture.

Graduating in medicine at Trinity College, Dublin, in 1924, where he was awarded the Vice-Chancellor's prize in English prose, he was appointed House Surgeon at the General Hospital, St John's, Newfoundland. He became interested in the deficiency diseases of the poorer fishermen and published an article on vitamin A deficiency in 1928 and a comprehensive report, 'Beriberi and other Deficiency Diseases in Newfoundland and Labrador', in the *Journal of Hygiene* in 1930. This 30-page report illustrates well the qualities which were to become the hallmark of his work—a broad approach, clear presentation of facts and a deep compassion for the unfortunate victims of malnutrition. He recognised that the diseases found were not due to deficiencies of single nutrients but multiple deficiencies due to poverty and a monotonous winter diet. His findings were used, and confirmed, by a team, which included B. S. Platt and W. H. Sebrell, conducting a medical survey of nutrition in Newfoundland some fifteen years later.

After his appointment to the Health Organisation of the League of Nations in 1931 he pursued his research into the link between malnutrition and poverty at a time of international recession and severe unemployment. His report, *Diet in Relation to Small Incomes*, published by the League of Nations in 1933, included a scholarly discussion on adequate dietary standards and satisfactory nutrition at low cost and his critical appraisal and distrust for the high protein allowances recommended was well in advance of his time and makes salutary reading today. The report is a valuable historical record of the time, containing studies of working class diet and expenditure on food in Germany, England and the USA.

The Health Organisation of the League of Nations decided, in 1934, that a general report on nutrition was needed after some ten years of studies of nutrition in the field of public health. Dr Aykroyd and Dr Etienne Burnet were asked to undertake this by making a series of enquiries in different countries and to report on nutritional status, research and policies. Their publication, *Nutrition and Public Health*, printed by the League of Nations in 1935, was an important statement of the current nutrition situation and was used extensively by international committees in public health and agriculture as well as the International Labour Office. Following this report the Health Organisation

set up a Technical Commission for the Study of Nutrition which convened meetings of groups of experts who then produced reports (a procedure continued by FAO and WHO). The first report, *The Physiological Bases of Nutrition* (1936), relied heavily on the findings of the Aykroyd and Burnet report.

Following his appointment as Director of the Nutrition Research Laboratories in Coonoor, India, in 1935, Dr Aykroyd continued his work as an expert for the Technical Commission including research on the effect on nutrients of the milling of cereals and the influence of climate on food requirements. His work in India greatly enhanced the reputation of nutrition research in that country and the world famous Indian National Institute of Nutrition at Hyderabad was the result of his efforts. To this day, revised editions of *The Nutritive Value of Indian Foods and the Planning of Satisfactory Diets* retains his name as principal author in recognition of his original concepts for the work.

During his period at FAO from 1946 Dr Aykroyd's leadership gave the Nutrition Division a fine start and the reports of the Expert Committees (some jointly with WHO), which owed a lot to his guidance and editing, are consulted throughout the world. After his retirement from FAO, in 1960, he joined Professor B. S. Platt at the Department of Human Nutrition, London School of Hygiene and Tropical Medicine, where, as Senior Lecturer, he helped to set up regular courses which led to the present MSc in Human Nutrition, attended by graduates from all over the world. He wrote several books at this time including *Food for Man and The Conquest of Deficiency Diseases* which explained nutritional problems in terms understandable and interesting for the untrained reader. During his work as co-author of the FAO Studies, *Legumes in Human Nutrition and Wheat in Human Nutrition* he was able to indulge in his love of history as well as use his powers of marshalling a mass of facts into lucid and readable prose.

This interest in history, which had led to the writing in 1935 of *Three Philosophers*, one of whom had been his great hero, Lavoisier, was also evident in his book *Sweet Malefactor: Sugar, Slavery and Society*.

During his active retirement he carried on with nutrition consultancy work for WHO and OXFAM and found time to write *The Conquest of Famine*. A fitting tribute was made when this received the first award of a joint book award scheme of the British, Swedish and American Nutrition Foundations.

Joyce Doughty



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# announcements

## Appointments

The National Academy of Sciences has elected 15 distinguished scientists from seven countries as foreign associates of the Academy: **Neil Bartlett** (UK), professor of chemistry, University of California, Berkeley, California; **Donald O. Hebb**, chancellor (retired), McGill University, Montreal, Canada; **John R. Hicks**, professor emeritus, University of Oxford, All Souls College, Oxford, UK; **Sir Andrew F. Huxley**, Foulerton research professor of the Royal Society, University College, London, UK; **Pierre Joliot**, chef de service, Institut de Biologie Physico-Chimique, Paris, France; **Michael S. Longuet-Higgins**, Royal Society research professor, department of applied mathematics and theoretical physics, University of Cambridge, UK; **Mary Lyon**, head, genetics section, Medical Research Council Radiobiology Unit, Berkshire, UK; **Digby Johns McLaren**, director general, Geological Survey of Canada, Ontario, Canada; **Sir Gustav J. V. Nossal**, director, Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia; **Alan Robertson**, deputy chief scientific officer, Agricultural Research Council, Animal Breeding and Genetics Research Organisation, Edinburgh, UK; **Abdus Salam**, professor of physics, Imperial College, London, UK; **Jean-Pierre Serre**, chair of algebra and geometry, College de France, Paris, France; **Roger Y. Stanier**, professor, department of biochemistry and microbial genetics, Institut Pasteur de Paris, Paris, France; **Cornelius A. G. Wiersma** (Netherlands), professor of biology, California Institute of Technology, Pasadena, California; **Yakov B. Zeldovich**, academician, Institute of Applied Mathematics, Academy of Sciences, Moscow, USSR.

The Royal Society has elected the following as Foreign Members of the Society: **Dr Julius Axelrod**, Chief of the Section of Pharmacology at the National Institute of Mental Health, Bethesda; **Professor Joshua Lederberg**, President of the Rockefeller University, New York; **Professor Jerzy Neyman**, Professor and Director of the Statistical Laboratory at the University of California, Berkeley; **Professor Yakov Borisovich Zeldovich**, Professor at the Institute of Chemical Physics in the Academy of Sciences, Moscow, USSR.

The Council of the Royal Society has appointed **Professor R. K. O'Nions** to a Royal Society Research Professorship under the scheme established in 1962 with support from HM Government for the Royal Society Research Professorships in universities in the UK.

**Dr Austin Woodland**, CBE, the present Director of the Institute of Geological Sciences, is to retire at the end of May this year and the Natural Environment Research Council has appointed **Professor G. M. Brown**, at present Professor of Geology at the University of Durham, to succeed him.

**Dr Patrick Grove**, Group Managing Director of the Radiochemical Centre is retiring on 16 May 1979. He will be succeeded by **Dr J. Stuart Burgess** who has been made a director of the company from 3 May 1979.

**Sir Charles Pereira**, formerly chief scientist, Ministry of Agriculture, Fisheries and Food, has been appointed Royal Society Inter-University Council Visiting Professor at the University of Nairobi, under a scheme to help scientific interchange between the UK and the Commonwealth.

**J. G. Dawson**, Chairman and Managing Director of the Zenith Carburettor Company Limited, has been elected the new President of the 72,000-member Institution of Mechanical Engineers.

The Agricultural Research Council has approved the appointment of **Professor John Postgate** as Director of the Unit of Nitrogen Fixation, University of Sussex, when the present Director, Professor Joseph Chatt, retires in March 1980.

The Governing Body has appointed **Dr Peter R. Day** to become Director of the Plant Breeding Institute from 2 July 1979.

**Dr John P. Hearn** has been appointed Director of the Wellcome Laboratories of Comparative Physiology, Institute of Zoology, The Zoological Society of London and will take up his appointment on 1 September 1979.

**Dr R. W. Nesbitt**, Reader in Geology at Adelaide University, has been appointed to a Chair of Geology with effect from 1 September 1979.

**Dr David J. Sherratt** has been appointed to the Chair of Genetics in the University of Glasgow with effect from 1 April 1980.

**Mr Christopher Ian Pogson**, at present Lecturer in Biochemistry in the University of Kent at Canterbury, has been elected to a Chair of Biochemistry at the University of Manchester.

**Dr Robert M. Farr**, Lecturer in Social Psychology at University College London, has been appointed to the newly established second Chair in Psychology in the University of Glasgow from 1 October 1979.

The British Committee of Award announces the following appointments to Harkness Fellowships of the Commonwealth Fund: **P. H. Allen** (Freshfields, and Exeter College, Oxford) Business Administration; **C. R. Bean** (H.M. Treasury) Economics; **Miss A. Campbell** (Department of Psychology, University of Oxford) Psychology; **H. J. Davies** (H.M. Treasury) Management Studies; **Miss J. Dodd** (King's College, Cambridge) Neurobiology; **Miss R. Elias** (BBC Television) Journalism; **P. J. Fray** (Clare College, Cambridge) Physiological Psychology; **L. N. Goldman** (Jesus College, Cambridge) American History; **D. M. Herman** (Trinity College, Cambridge) Literature; **H. D. Keelan** (Queen's College, Cambridge) Music; **Miss S. Laird** (St Anne's College, Oxford) Soviet Studies; **S. F. Lee** (Balliol College, Oxford) Law; **G. A. M. Leggatt** (King's College, Cambridge) Philosophy of Law; **Miss P. Lund** (Department of Physiology, University of Newcastle upon Tyne) Endocrinology; **J. N. Pearson** (Department of the Environment) Public Administration; **R. J. Peel** (Arthur Andersen & Co.) Business Administration; **E. Powell** (Pembroke College, Oxford) Legal History; **M. F. Scanlon** (Royal Victoria Infirmary, Newcastle upon Tyne) Endocrinology; **P. A. Swaab** (Christ's College, Cambridge) American Literature; **J. Watkins** (Hammersmith Hospital, Royal Postgraduate Medical School) Medicine.

## Awards

**Finlay MacRae**, District Officer with the Forestry Commission, has won an international conservation award for his work in conserving the native Caledonian Pinewoods of Glen Affric. The award is made by the Society of American Travel Writers.

**Dr Greg Clark**, a Principal Research Scientist with the Division of Mineral Physics in Sydney, has been awarded the Pawsey Medal for distinguished research in experimental physics.

The André Lichtwitz prize (10,000 French Francs) is awarded every year by INSERM ("Institut National de la Santé et de la Recherche Médicale") for distinguished clinical or fundamental research on calcium and phosphorus metabolism carried out by a French or foreign scientist (or a team). Applicants who have previously applied are allowed to apply again. Applications: Director of the INSERM (c/o Mlle C. Chirol, 101 rue de Tolbiac, 75645-PARIS CEDEX 13, France) by 30 June 1979.

The Clarke Institute of Psychiatry, Annual Research Fund Award is awarded annually to a clinical or basic scientist who has published a report or dissertation on outstanding research within the field of mental health conducted during the preceding year. The scientists must have carried out his work in Canada while resident in Canada. He may apply or be nominated for the prize up to 1 June in the year following publication. Applications: J. E. Stewardson, Secretary, Research Fund Committee, Clarke Institute of Psychiatry, 250 College Street, Toronto, Ontario Canada M5T 1R8.

## Meetings

7 June, **Conference on Forestry Policy**, London (The Royal Society of Arts, 8 John Adam St, Adelphi, London WC2).  
12-14 June, **Fuel Economy and Emissions of Lean Burn Engines**, London (The Institution of Mechanical Engineers, 1 Birdcage Walk, London SW1, UK).  
12-13 June, **Rural-Urban Interchange for Progress**, London (The Overseas Development Ministry, Eland House, Stag Place, London SW1, UK).  
18-20 June, **Engineering Aspects of Magnetohydrodynamics**, Butte (D. R. Brown, 18th MHD Symposium, Merdi, PO Box 3809, Butte, Montana 59701).  
20 June, **Experience of Cathodic Protection in Steam and Process Plant**, London (The Institution of Mechanical Engineers, 1 Birdcage Walk, London SW1, UK).  
29 June, **Inflammation Mechanisms, Therapy and Investigative Techniques**, Glasgow (Dr I. J. Zeitlin, Dept of Physiology and Pharmacology, University of Strathclyde, George St, Glasgow, UK).  
5-7 July, **Scientific Criteria and Methods for Drug Assessment**, Rome (Fondazione Giovanni Lorenzini, Via Monte Napoleone, 23, 20121, Milan, Italy).

9-13 July, **The Mechanisms of Reactions in Solutions**, Canterbury (Dr J. F. Gibson, The Chemical Society, Burlington House, London W1, UK).  
10-11 July, **Noise and Vibration of Engines and Transmissions**, London (Institution of Mechanical Engineers, 1 Birdcage Walk, London SW1, UK).  
12-13 July, **Dietary Management of Disease**, London (Dr M. R. Turner, British Nutrition Foundation, 15 Belgrave Square, London SW1, UK).  
15-17 August, **20th Canadian High Polymer Forum**, Quebec (Dr Ian McEwan, CIL Paint Research Laboratory, 1330 Castlefield Ave, Toronto, Ontario, Canada M6B 4B3).  
29-31 August, **4th International Symposium on Mycotoxins and Phycotoxins**, Lausanne (Prof. Palle Krogh, Dept of Veterinary Microbiology, Purdue University, West Lafayette, Indiana 47907).  
12-14 September, **Charged-Coupled Devices**, Edinburgh (Mr W. Campbell, Centre for Industrial Consultancy and Liaison, University of Edinburgh, 16 George Square, Edinburgh, UK).  
16-21 September, **Advanced Immunoassay Workshop**, Guildford (Mr B. A. Morris, Division of Clinical Biochemistry, Dept of Biochemistry, University of Surrey, Guildford, Surrey, UK).  
18-21 September, **The Economics of Information**, Brighton (Aslib, 3 Belgrave Square, London SW1, UK).  
26-28 September, **New Coal Upgrading Processes**, Luxembourg (Commission of the European Communities, Bureau d'Organisation et de Coordination des Conférences, Bâtiment Jean Monnet, B1/32, Plateau du Kirchberg, Luxembourg).  
28 September, **Practical Applications of Microwave Energy**, Manhattan (Dr D. Y. C. Fung, Call Hall, KSU, Manhattan Kansas 66506).  
1-3 October, **10th Annual Underwater Mining Institute**, Galveston (Prof. J. Robert Moore, Marine Science Institute, The University of Texas, PO Box 7999, Austin, Texas 78712).  
1-3 October, **Gesellschaft für Immunologie**, Innsbruck (Prof. Dr G. Wick, Institute for General and Experimental Pathology, Fritz-Pregl-str. 3, 6020 Innsbruck, Austria).  
1-3 October, **2nd International Recycling Congress**, Berlin (CRE/MER, PO Box 33002 10, D-1000 Berlin 33, FRG).  
2-4 October, **Grain Dust—Its Characteristics, Explosibility, Hazard Control and Utilization**, Manhattan (Dr B. S. Miller, US Grain Marketing Research Laboratory, 1515 College Ave, Manhattan, Kansas 66502).  
3-6 October, **1st International Congress on Hormones and Cancer**, Rome (S. Lacobelli, Laboratorio di Endocrinologia Molecolare, Istituto di Clinica

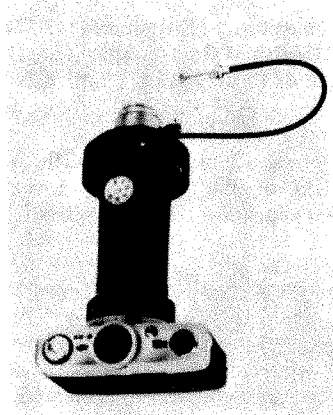
Osterrica e Ginecologica, Università Cattolica del S. Cuore, Via della Pineta Sacchetti, 644, 00168, Rome, Italy).  
3-6 October, **Hormones and Cell Regulation**, Strasbourg (Dr J. Nunez, Unite de Recherche sur la Glande Thyroïde, Inserm, 79 Ave du General Leclerc, 94 Bicetre, France).  
9 October, **Testing the Body**, London (Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).  
4-18 October, **Latent and Persistent Virus Infections in Man**, Israel (KENES, 8 Shmuel Hanagid St, PO Box 983, Jerusalem, Israel).  
9-11 October, **Progress and Problems in Radioelement Analysis**, Gatlinburg (Public Relations Dept, Union Carbide, Nuclear Division, PO Box Y, Oak Ridge, Tennessee 37830).  
15-18 October, **Weather and Air Pollution**, Scarborough (National Society for Clean Air, 136 North St, Brighton).  
16-17 October, **Quantitative Surface Analysis**, Teddington (Dr C. Lea, Division of Chemical Standards, National Physical Laboratory, Teddington, Middlesex, UK).  
22-25 October, **RECLAN 79**, Eastbourne (The Conference Secretariat, Society of Chemical Industry, 14 Belgrave Square, London SW1, UK).  
28-31 October, **10th Yugoslav Symposium on Biophysics**, Radenci (Gregor Cevc, J. Stefan Institute, Jamova 39, PO Box 199, 61001 Ljubljana, Yugoslavia).  
28 October-2 November, **Water Chlorination: Environmental Impact and Health Effects**, Colorado (Public Relations Dept, Union Carbide Nuclear Division, PO Box Y, Oak Ridge, Tennessee 37830).  
29 October-3 November, **12th Annual Meeting of the American Association of Stratigraphic Palynologists**, Dallas (H. M. Simpson, Geoscience Research, ARCO Oil and Gas Company, PO Box 2819 (EXP-1060, Dallas, Texas 75221).  
2-4 November, **Machining**, Bournemouth (The Meetings Secretary, The Institution of Metallurgists, Northway House, Whetstone, London N20, UK).  
13 November, **Significance of Defects in the Failure of Fibre Composites**, London (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).  
13-15 November, **Post-Harvest Crop Conservation**, Harrogate (Dr R. Gordon, 19 Homewood Road, St Albans, Herts, UK).  
27-30 November, **Forage Conservation in the '80s**, Brighton (The Conference Secretariat, British Grassland Society, The Grassland Research Institute, Hurley, Maidenhead, Berks, UK).  
26-29 November, **4th World Ozone Congress**, Houston (R. S. Croy, International Ozone Association, 14805 Detroit Ave, Cleveland, Ohio 44107).



# newly on the market

**Agarose gel.** LKB introduce the LKB Agarose-EF—a new type of agarose with very low electroendosmosis, and the new 1802 Ampholine carrier ampholytes for electrofocusing in agarose. These open a wide range of new application areas for electrofocusing.  
**Circle No. 43 on Reader Enquiry Card.**

**Camera back.** Bausch and Lomb have announced a new 35-mm camera back for Series II photomicrographic equipment and the AX-1 automatic exposure controller. The camera back fits all current focusing tubes and includes 3× and 5× adaptors. An automatic dark slide is incorporated which opens when the camera back is snapped into the adaptor on the focusing tube, and closes automatically when the back is removed. An opening in the camera back accepts a plastic strip on which pertinent data can be recorded. The data are then photographed along with the slide to simplify photographic identification. The camera back is par-focussed and par-centred with the microscope eyepieces.  
**Circle No. 44 on Reader Enquiry Card.**



Bausch and Lomb camera back

**Portable container.** Laboratory Impex. Magic-Temp is a fully portable container designed to transport samples or other material safely and at the correct temperature. The unit will work on normal mains electricity or from the cigarette lighter socket of a car or van. Magic-Temp operates in 3 modes: as a refrigerator, a freezer and an incubator. It has a deep drawn heavy duty aluminium inner chamber, foam-in-place polyurethane insulation and an exterior of high impact plastic. The capacity of Magic-Temp is more than 14 l, total weight 13 kg.  
**Circle No. 45 on Reader Enquiry Card.**

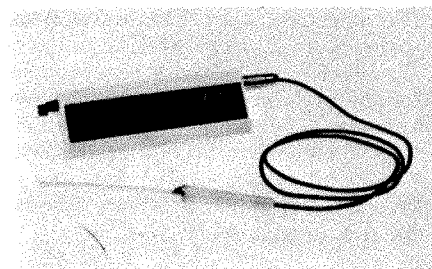
**Calibration aid** for microscopists. Dunn and Reidman. The Photomicrograph Scale Marker from Dunn and Reidman is designed on a slide-rule principle, and is used for the direct application of any desired size of scale mark onto photomicrographs. The user simply selects a scale mark and pulls out the inner slide to correspond with the photograph magnification, thus automatically setting the instrument to produce the selected scale mark. Another function of this instrument is that of measuring the real-life size of structures directly from photomicrographs. The Photomicrograph scale marker is constructed from high-quality plastic and is supplied in a protective pouch.  
**Circle No. 46 on Reader Enquiry Card.**

**Diffusion zone reader.** Dynatech. The improved diffusion zone reader from Dynatech will accept bioassay plates up to 25 cm<sup>2</sup> (twice the size of earlier models). Designed for the measurement of precipitin rings and antibiotic sensitivity zones, the machine can also be used in electrophoretic and immunodiffusion studies. A clear direct optical image of the zone to be measured is provided. The object is lit by dark ground illumination and the measuring cursor lines are projected by a top-illumination system so that they are superimposed onto the zone. Simple adjustments can be made to the optical system to compensate for parallax shift and to allow for variations in the heights of different objects.  
**Circle No. 47 on Reader Enquiry Card.**

**Laboratory freeze drier.** ChemLab. The model SB3 is a compact freeze drier which incorporates a built-in refrigeration system. The SB3 is normally fitted with a manifold to which laboratory glassware can be attached. The condenser chamber has an ice capacity of 3 l. The coil is chilled by the circulation of Freon 502 from the refrigeration unit. This model is normally supplied complete with a manifold and the flange of the manifold is connected to the side of the condenser. The vacuum pump for use with the model SB3 should have a displacement of about 50 l min<sup>-1</sup>.  
**Circle No. 48 on Reader Enquiry Card.**

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Enquiry Card bound inside the cover.

**Digital thermometers.** Digical announce a range of portable digital thermometers for medical and scientific use. These thermometers have the advantage of permanently pre-set calibration, interchangeability of all probes, and fast response (<15 s in certain circumstances). A range of 25 probes is available. Options available include: model DC-1 thermometer—range -50 °C to +150 °C (resolution 0.1 °C); model DC-M thermometer—range +30.00 °C to +49.99 °C (resolution 0.01 °C). Multi-input unit for differential application; alarm units; readout option in °F are available additional features.  
**Circle No. 49 on Reader Enquiry Card.**



Digital portable thermometer

**Carcinoembryonic antigen control serum.** Bio-Rad. Carcinoembryonic antigen (CEA) control serum (human) is now available from Bio-Rad Laboratories. This material is manufactured from pooled human serum and offers the user two levels of the antigen. Level 1 provides CEA representative of the high normal range (2.5–5.0 ng ml<sup>-1</sup>) while level 2 represents the range clinically significant for malignancies (10–20 ng ml<sup>-1</sup>). CEA control serum is a stable, lyophilised control serum and contains no preservatives, stabilisers or other catalytic or non-reactive ingredients.  
**Circle No. 50 on Reader Enquiry Card.**

**High performance GC/MS system.** Varian. The new MAT 212 is a high performance GC/MS system. Its resolution is >20,000 (10% valley), the accuracy of mass determination better than 3 p.p.m. The basic system includes a dual-column Varian 3700 gas chromatograph. The link between Varian 3700 and mass spectrometer is an open split coupling for capillary and packed columns. A single stage all-glass jet separator for packed column work is optional.  
**Circle No. 51 on Reader Enquiry Card.**

**Automatic sputter coater** for scanning electron microscopy. Nanotech. Advantages of the Semprep 2 sputter coater from Nanotech for routine EM preparation include a degree of automation built into the standard equipment that ensures reproducible results without the need to set up controls for each deposition, and cleanliness guaranteed by the integral two-stage rotary vacuum pump and associated metal pipework. A built in electron deflection system and associated specimen cooling prevent thermal damage to sensitive specimens, and a closed loop system controls the sputtering current and voltage. The sputtering current required is selected and held constant electronically. The sputtering voltage is selected and is then held constant by closed loop control of the argon gas inlet valve.

**Circle No. 52 on Reader Enquiry Card.**



The Nanotech Semprep 2 sputter coater

**IR Spectrophotometers.** Perkin-Elmer. The microprocessor controlled Model 98 series consists of three instruments: the Model 598, scanning from 4,000  $\text{cm}^{-1}$  to 200  $\text{cm}^{-1}$ , the Model 398, scanning from 4,000  $\text{cm}^{-1}$  to 400  $\text{cm}^{-1}$ , and the Model 298, from 4,000  $\text{cm}^{-1}$  to 600  $\text{cm}^{-1}$ . There is a choice of three slit programs and six scan times. A five-digit wavenumber display allows the monochromator to be set accurately and reproducibly to any chosen frequency. The chart and monochromator drives are synchronised by the microprocessor. Other standard features include repeat scan facilities, wavenumber limits, choice of chart formats and rapid indexing of both chart and monochromator to 4,000  $\text{cm}^{-1}$ .

**Circle No. 53 on Reader Enquiry Card.**

**Monoclonal anti-Thy 1.2.** New England Nuclear (NEN) has announced the availability of a sensitive tool in cellular research, monoclonal anti-Thy 1.2, for identification, localisation and selective isolation of mouse T-cell lymphocytes. This high-titre anti- $\theta$  antibody is extraordinarily specific for the Thy 1.2 antigen on the surface of mouse T-cell lymphocytes. It is offered in two concentrations: as  $\sim 1$  mg immunoglobulin, or  $\sim 0.1$  mg immunoglobulin, both supplied in 1 ml of buffer system.

**Circle No. 54 on Reader Enquiry Card.**

**Balances.** Mettler. The PL/SE range of balances from Mettler has been designed specifically for hot cell use. Only the weighing cell is located in the radioactive or sterile environment—the electronics which convert the weight readings to a digital form are placed outside. The rugged construction of the three PL/SE balances makes them suitable for use in highly radioactive surroundings and the 5-m long connection obviates the need for a separate power supply in the weighing cell. The PL200SE, PL1200SE and PL3000SE offer a weighing range of 220–0.001 g, 1,200–0.01 g and 3,500–0.1 g, respectively.

**Circle No. 55 on Reader Enquiry Card.**

**pH Electrodes.** MSE. Four 'unbreakable' pH electrodes, a pH meter, and a new type of automatic temperature compensation probe have been introduced by MSE Scientific Instruments. The model 9115 semi-micro electrode for measurements in test tubes is 6 mm in diameter. The model 9125 is 1 ft long. The model 9105 is a standard combination pH electrode, while the model 9135 has a flat sensing tip for measurements of pH on surfaces. All electrodes are permanently gel-filled combination electrodes, with tough epoxy bodies and they can be supplied with a variety of plugs. Also new is the model 501 pH meter; pH to the nearest 0.01 units and automatic temperature compensation probe which enables the meter to be used also as a digital thermometer.

**Circle No. 56 on Reader Enquiry Card.**

**Dewar flasks.** Day-Impex have introduced two additions to their 'Dilvac' range of glass Dewar flasks in stainless steel containers. The first is a 7-l size, which has toggle-clamps to secure the lid, a strong carrying handle and a cushioned base. On this model there is also a handle on the lid, and the wide diameter base of the container creates a very low centre of gravity, ensuring safe transportation of specimens etc. The second new style is a range of wide-mouthed shallow form Dewars in four sizes. Borosilicate glass is used as an inner vessel for the robust stainless steel casing. The wide mouths are designed so that boiling flasks of four sizes, 100, 250, 500 and 1,000 ml, will fit easily.

**Circle No. 57 on Reader Enquiry Card.**

**Plasma chemistry equipment.** Nanotech have now developed their range of plasma chemistry equipment to allow fully automatic load tuning in conjunction with closed loop control of the RF power levels. Automatic controls allow unattended operation for long reaction periods and also operation by unskilled personnel. The applications of plasma chemistry in-

clude organic removal and sample preparation for atomic absorption spectroscopy.

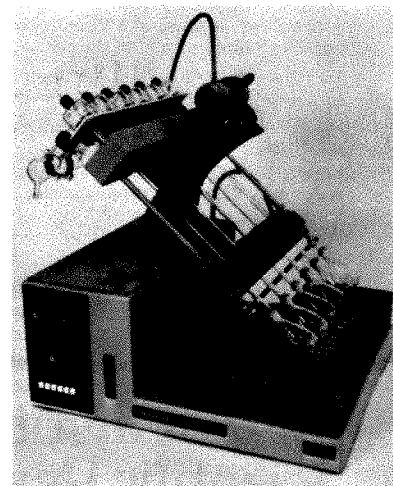
**Circle No. 58 on Reader Enquiry Card.**

**Data analyser.** Du Pont. The MWD-1 from Du Pont is a compact data collection and analysis system for automatic acquisition, reduction, and printout of molecular weight distribution (MWD) data. This system eliminates the manual calculations usually associated with chromatographic polymer characterisation techniques. Total high performance size exclusion chromatography analyses in less than 10 min are routinely possible. The MWD-1 automatically adjusts raw chromatographic data for column dispersion and reports corrected molecular weight results. Another feature is on-line calibration with either narrow or broad base standards. Calibration routines can be entered into the system and stored in memory via magnetic programming strips.

**Circle No. 59 on Reader Enquiry Card.**

**Digester to determine trace elements.** Büchi. The Büchi digesting system digests organic and biological materials in a closed quartz apparatus. The main ranges of application lie in the analyses of foodstuffs and feedstuffs, clinical-medicinal chemistry and ecology. The system is automatic and can be operated by semi-skilled personnel. Digestion under total reflux in a closed system prevents foreign elements being carried in. The acid consumption is very low. The controls permit manual as well as automatic operation. The glass components of the six digestion points are interchangeable. The apparatus has 100-ml flasks as standard equipment. Six tests can be carried out simultaneously. Weighed samples of up to 30 g can be used. The digesting temperature is selectable between 150 and 360 °C.

**Circle No. 60 on Reader Enquiry Card.**



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## APPOINTMENTS VACANT

### UNIVERSITY OF KHARTOUM—SUDAN

Applications are invited for the post of  
**LECTURER**

**IN MEAT PRODUCTION**

in the

**DEPARTMENT OF  
ANIMAL PRODUCTION**

Salary scale (exclusive of cost of living allowance): £51,500 to £53,500 per annum (£51=£1.23 sterling). The British Government may supplement salary in range £3,894 to £4,542 per annum (sterling) for married appointee or £1,830 to £2,310 per annum (sterling) for single appointee (reviewed annually and normally free from tax) and provide children's education allowances and holiday visit passages. Family passages; baggage allowance; superannuation scheme; unfurnished accommodation available; various allowances.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct by air mail to Acting Personnel Secretary, University of Khartoum, P.O. Box 321, Khartoum, Sudan, by July 18, 1979.

Applicants resident in the U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 1098(A)

### Re-advertisement UNIVERSITY COLLEGE OF NORTH WALES

Applications are invited for the post of  
**LECTURER**

**IN AGRICULTURE**

with particular responsibilities for Grassland Production. Applicants should have a degree in Agriculture, Agricultural Science, or a related discipline with a sound basis in Agronomy.

Applicants should be interested in developing research in the pasture-animal relationships with reference to the efficiency of utilisation of grassland, and in helping to co-ordinate the activities of a developing team interested in the wider field of Animal Production from Grassland.

The appointment, to commence on September 1, 1979, or any earlier date by agreement, will be to the Universities' Lecturer scale: £4,232 to £8,452 per annum.

Applications (two copies), giving details of qualifications and experience, together with the names and addresses of three referees, should be sent to the Assistant Registrar (Personnel), University College of North Wales, Bangor, Gwynedd LL57 2DG, from whom further particulars may be obtained.

Closing date for applications: June 25, 1979. 2023(A)

### New Zealand UNIVERSITY OF CANTERBURY Christchurch SENIOR LECTURER/ LECTURER

**IN COMPUTER SCIENCE**

Applications are invited for the above-mentioned position. Applicants should have interests within the (very broad) areas of computer systems and languages, theory of computing, information systems, or other areas of non-numerical applications.

The salary for Lecturers is in a scale from NZ\$11,894 to NZ\$14,615 per annum. Promotion may be made on academic merit to Senior Lecturer where the salary range is from NZ\$14,983 to NZ\$17,145 (bar) NZ\$18,665 per annum.

Applications close on August 15, 1979.

Particulars, including information on travel allowances, study leave, housing and superannuation, may be obtained from the Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF. 1097(A)

### GEOCHRONOLOGIST

A Geochronology Laboratory is to be established at the University of Regina in the Department of Geology. The successful applicant will be required to design, set-up and operate a solid source mass spectrometry laboratory specialising in uranium-lead and zircon dating methods. The position will be of particular interest to persons with a strong physics background. A knowledge of solid state electronics, minicomputers and data handling. The position, which does not require any teaching or administrative duties, will commence as soon as a suitable candidate is found. Salary will be commensurate with qualifications and experience. Applications, which must indicate availability and salary expected, should be forwarded, along with three letters of reference, to Dr G. R. Parslow, Department of Geology, University of Regina, Regina, Saskatchewan, S4S 0A2, Canada (PRIOR TO JUNE 30, 1979). W121(A)

### NATIONAL VEGETABLE RESEARCH STATION ASSISTANT PLANT BREEDER

A SCIENTIFIC OFFICER is required to assist in research into onion and carrot breeding.

The appointment will be in the grade of Scientific Officer scale from £2,839 rising by annual increments to £4,415.

Minimum qualification required is a Degree in biological science or applied biology.

Non-contributory employers' additional pension scheme.

Full particulars and application form (to be returned by June 19, 1979) from the Secretary, National Vegetable Research Station, Wellesbourne, Warwick CV35 9EF. 1014(A)

### CHARING CROSS HOSPITAL MEDICAL SCHOOL (University of London) TWO RESEARCH ASSISTANTS

Applications are invited from graduates with First or Second class degrees in a biological science for the above posts, one tenable in the Department of Microbiology at Charing Cross Hospital Medical School and the other at the Institute of Obstetrics & Gynaecology at Queen Charlotte's Maternity Hospital, W.6. Work will be on vertical transmission of cytomegalovirus. Previous experience of virological techniques is not essential.

Salary £3,486 to £3,798 per annum plus £354 London Weighting Allowance (under review).

Further details obtainable from Dr. J. C. Coleman, 01-748 2040 extension 2499, and applications on forms from the School Secretary, Charing Cross Hospital Medical School, The Reynolds Building, St. Dunstan's Road, London, W6 8RP. (2030)A

### UNIVERSITY OF RHODESIA DEPARTMENT OF GEOGRAPHY LECTURESHIP IN GEOGRAPHY

Applications are invited for the above-mentioned post. Candidate should indicate in their applications the full range of their interests and abilities as well as their special field of study. Preference may be given to persons whose special field of study is Geomorphology (with Biogeography).

**Salary Scales (Approx. Sg. Equiv.)** Lecturer Gr. I £7,021 by £264 to £8,077; Lecturer Gr. II £4,334 by £221 to £5,474 by £264 to £6,794. Both permanent, pensionable terms and short-term one or two-year contract are offered.

**Permanent, Pensionable Terms.** Family passages and allowance towards transport of effects on appointment. Installation loan of up to half of one year's salary if required. University accommodation guaranteed for a period of at least three years for persons recruited from outside Rhodesia. Sabbatical leave and triennial contact visits with travel allowance. Superannuation and medical aid schemes.

**Short-term Contracts:** Family passages and allowance towards transport of effects. Assistance with accommodation for persons recruited from outside Rhodesia.

**Applications:** (6 copies) giving full personal particulars (including full names, place and date of birth, etc. qualifications, experience and publications, and names and addresses of three referees, should be submitted to the Registrar, University of Rhodesia, P.O. Box MP 167, Mount Pleasant, Salisbury, Rhodesia. Overseas applicants should send an additional copy of their applications to the Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1 0PF. Further particulars may be obtained from either of the above.

Closing date for receipt of applications: June 30, 1979.

British subjects considering application for posts in Rhodesia are urged to consult the Foreign and Commonwealth Office (telephone 233 8727) or their nearest British Consular Office. 2026(A)

## Researcher

### Overseas Assignment

The Kuwait Institute for Scientific Research, a rapidly expanding institute for applied science and technology, is seeking a Researcher interested in a career challenge.

You should have a Ph.D. in Chemical Engineering or Civil Engineering with a minimum of 3 years' experience in Mix Design Core Analysis for road constructions.

Kuwait Institute offers attractive salaries commensurate with qualifications and experience, furnished housing, liberal fringe benefits including round trip air tickets and free medical benefits.

Please airmail your complete resume by July 25, 1979 to: Mr. Habib Al-Sahhaf, Personnel Manager, Kuwait Institute for Scientific Research, P.O. Box 24885, Safat, Kuwait, State of Kuwait. W144(A)

## Kuwait Institute for Scientific Research

**THE RESEARCH ORGANIZATION OF SYNTHELABO**  
an internationally recognized European Pharmaceutical Group located in Paris

*requires a*

## SENIOR PHARMACOLOGIST IN METABOLIC DISEASES

to lead the Group of Intermediary Metabolism in the Department of Biology

The high success rate and rapid growth of this pharmaceutical group is related to the quality of people they employ and their continued commitment to research and development.

In order to succeed in this challenging and important post, the successful applicant, either male or female, must be capable of leading a team of graduates and technicians and would be responsible for both the fundamental and applied work of the group. The group is actively researching into anti-obesity and anti-hyperlipidaemic drugs and has an increasing interest in anti-diabetic drugs.

Applicants should be a PhD Biochemical Pharmacologist with at least five years post doctoral experience and preferably with industrial experience as well.

A realistic salary can be expected and relocation assistance will be available with other financial benefits.

All applications will be treated in the strictest confidence and should be sent directly to Mr. J. Brault at the following address:

**SYNTHELABO,  
58, Rue de la Glaciere,  
75013 Paris.**

**W154(A)**

**THE LONDON HOSPITAL  
MEDICAL COLLEGE  
(University of London)  
DEPARTMENT OF BIOCHEMISTRY  
JUNIOR LABORATORY  
SCIENTIFIC OFFICER/  
LABORATORY  
SCIENTIFIC OFFICER**

required for general duties which will include some research work.

Salary on Whitley Scale £2,259 to £3,344 per annum, including London weighting, according to age and experience. Four weeks annual holiday plus extra days when College is closed.

For further details from Dr C. W. Parr, Tel: 047 0644, ext. 20. Applications to the Secretary, The London Hospital Medical College, Turner Street, London E1 2AD, quoting reference B/5/79. 1092(A)

**ROYAL POSTGRADUATE  
MEDICAL SCHOOL  
(University of London)  
DEPARTMENT OF  
CLINICAL PHARMACOLOGY  
SENIOR  
RESEARCH OFFICER  
(Postdoctoral)**

required for clinically orientated research on Prostacyclin. Applicants may be chemists, biochemists, or pharmacologists as both biological and GC-MS work is in progress. Prostaglandin experience is desirable but not essential.

The appointment is for up to two years. Salary £4,232 plus £502 London Allowance or £4,505 plus £502 London Allowance in first year.

Application forms and further particulars may be obtained from the Personnel Office, Royal Postgraduate Medical School, 150 Du Cane Road, London W12 0HS, quoting ref. no. 20/202/N. 2001(A)

**FACULTY POSITION  
THE GRADUATE DEPARTMENT  
OF NUTRITION  
TUFTS UNIVERSITY**

has a faculty opening for a doctoral level person with teaching experience in animal as well as human nutrition, who will spend half time teaching students in the Department and in the School of Veterinary Medicine, and half time doing research.

Title, salary and types of research will be determined according to applicant qualifications.

Curriculum vitae and bibliography should be submitted to: Dr Stanley N. Gershoff, Chairman, Graduate Department of Nutrition; Director, Nutrition Institute, Tufts University, Medford, MA 02155.

Tufts University is an equal opportunity/affirmative action employer. W155(A)

**UNIVERSITY OF  
LONDON KING'S COLLEGE  
DEPARTMENT OF BIOPHYSICS  
PART- OR FULL-TIME  
TECHNICIAN**

Applications are invited for a post available immediately for a Technician with experience of either heteroduplex analysis using the electron microscope or restriction endonuclease mapping and sequencing of DNA.

The post is funded by a research grant and salary will be on Grade 5 scale, £3,998 per annum to £4,580 per annum inclusive.

Candidates should write with curriculum vitae and the names of two referees to: The Head Clerk (Ref: 217808/N), King's College, London, Strand, WC2R 2LS. 2065(A)

**KING'S COLLEGE HOSPITAL  
MEDICAL SCHOOL  
(University of London)**

Denmark Hill

London SE5 8RX

LIVER UNIT

Applications are invited for the position of

**RESEARCH ASSISTANT/  
FELLOW**

in this busy multi-disciplinary unit from candidates with experience in cell membrane biology, immunochemistry or protein biochemistry, to work on human liver cell surface antigens.

Salary will be on University of London scales, according to qualifications and experience.

Please apply, giving full curriculum vitae and the names and addresses of two referees to the Secretary of the Medical School at the above address, from whom further details may be obtained. Closing date: June 29, 1979. 2002(A)

**THE MIDDLESEX HOSPITAL  
MEDICAL SCHOOL  
(University of London)**

Applications are invited for the post of  
**RESEARCH ASSISTANT**

to work on the cellular basis of cartilage growth. This post will be supported by the Medical Research Council for three years, commencing October 1, 1979.

Salary up to £5,304 per annum (including London Allowance) depending on age and experience.

Applications, together with the names of two referees, to Professor L. Wolpert, Department of Biology as Applied to Medicine, The Middlesex Hospital Medical School, Cleveland Street, London W1P 6DB. 2067(A)

**WESTMINSTER  
MEDICAL SCHOOL  
RESEARCH ASSISTANT**

An organic Chemist or Biochemist is needed by a small team working on pigment changes in human disease. The post would be particularly suitable for a recent graduate or for someone graduating this year. The successful applicant would be required to develop methods for the isolation and assay of pigment intermediates from the body fluids of patients undergoing treatment and in the culture fluid from cells grown in tissue culture. Post available for one year initially.

Salary on the Whitley Council Scale for Hospital Biochemists.

Applications, with curriculum vitae and the names of two referees should be made to Professor J. R. Hobbs, Department of Chemical Pathology, Westminster Medical School, 17 Page Street, London SW1P 2AR. Closing date will be June 24, 1979.

Please quote: Reference KBC. 2027(A)

**Cancer Research Campaign  
Laboratories**

**UNIVERSITY OF  
NOTTINGHAM**

vacancy exists in the C.R.C. laboratories in Nottingham for a

**POSTDOCTORAL SCIENTIST**

to work on the biochemistry of tumour surface membranes with special reference to studies into the synthesis and turnover of tumour-associated antigens.

The post is offered for a period of 12 months from August 1979.

Applications in writing, quoting the names of two referees, to the Staff Appointments Officer, University of Nottingham, University Park, Nottingham NG7 2RD, not later than June 1979. Ref: 696. 1093(A)



## Leptospira Reference Laboratory

# Senior Grade Microbiologist

Applications for the above post are invited from graduate microbiologists with a wide experience in medical microbiology. The successful applicant will be required to assist with the routine work of the laboratory but in addition he/she will have the opportunity to contribute to the research and development programme.

Previous experience in microbiological research is essential, it need not have been in leptospirosis, although some knowledge of immunology would be an asset.

Salary scale £5,451 to £6,837 plus £354 London Weighting. N.H.S. Terms and Conditions of Service.

For further information write to: Dr J. D. Coghlan, Leptospira Reference Laboratory, Central Public Health Laboratory, Colindale, London NW9 5DX.  
2021(A)

**PH  
LS**

Public Health Laboratory  
Service Board.

## DEPARTMENT OF NEUROCHEMISTRY MAX-PLANCK-INSTITUT FÜR PSYCHIATRIE MUNICH

The Max-Planck-Gesellschaft offers

# YOUNG SCIENTISTS

the opportunity to set up their own research group in the above department.

Applicants should have already accomplished internationally recognised research within the general area of neurobiology, especially in membrane-receptor biochemistry, neurogenetics, neuroimmunology or neuroendocrinology.

Appointments will be limited to 5 years, the salary being within the scale AH3/C3. (about 50-55.000 DM per annum).

Each group will be able to grant post-doc-/doctoral scholarships to German and foreign scientists; it will also be provided with its own laboratory, inclusive technical staff, appropriate equipment and adequate running costs.

Applications should contain a tabular curriculum vitae, a list of publications, and a short description of scientific work both achieved and intended, and should be submitted to the

Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V.-Generalverwaltung-III a 4-Postfach 647, 8000 München 1, before September 30, 1979.  
W148(A)

## UNIVERSITY OF THE WITWATERSRAND Johannesburg

Applications are invited from suitably qualified persons for appointment to the following posts:

### DEPARTMENT OF GEOPHYSICS

#### SENIOR LECTURER/ LECTURER IN GEOPHYSICS SENIOR LECTURER/ LECTURER IN APPLIED GEOPHYSICS

The Department offers third-year level and Honours level courses in Geophysics, as well as the degree of B.Sc. (Eng.) in Applied Geophysics. The two appointees will be responsible for lectures, laboratory classes and supervision of research. Applicants could come from a range of professional disciplines but should have qualifications and experience in one or more of the major areas: geomagnetism, gravity, seismology, EM and galvanic methods, isotopes and heat flow, rock deformation science.

The salary ranges are:

SENIOR LECTURER: R11,400 to R15,600 per annum. LECTURER: R8,100 to R13,200 per annum (£1 equals R1.75 approx.).

### BERNARD PRICE INSTITUTE OF GEOPHYSICAL RESEARCH PHYSICIST/GEOPHYSICIST

The Institute seeks a physics or geophysics graduate for a post in its Isotope Geophysics laboratory. This laboratory undertakes geochronological and isotope evolution studies using the U-Pb, Rb-Sr, Sm-Nd methods and is equipped with two modern mass spectrometers utilising digital and computer facilities plus extensive ancillary facilities. The appointee would be responsible for maintenance and development of the mass spectrometers but would also become involved in a research project, the results of which could be used for gaining a higher degree. Some knowledge of digital and computer techniques is essential, but further training could be arranged; previous geological training would be advantageous but not essential. The salary will lie in the range of R5,850 to R13,200 per annum.

### STRUCTURAL GEOLOGIST

A geologist is required for field mapping and microscope study of deformed rocks in the Vredefort dome basement. A team is studying radial profiles, which probably extend 'down' a ~15 km-thick section of Archaean crystalline crust, into the middle crust. Applicants with an Hons. degree may use research results for a higher degree. The starting salary will lie in the range R4,950 to R9,000 per annum.

The appointees will be placed at an appropriate notch on the salary scales, according to qualifications and experience.

The University's policy is not to discriminate in the appointment of staff or the selection of students on the grounds of sex, race, religion, or colour. Further particulars relating to this practice and to the above posts are included in an information sheet obtainable from the London Representative, University of the Witwatersrand, Chichester House, 278 High Holborn, London WC1V 7HE, or from the Registrar, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, 2001 South Africa, with whom applications should be lodged. Applications in writing, giving full personal and career details, including telephone number, and the names and addresses of three persons who may be called upon to act as referees, should be lodged with the Registrar by July 6, 1979. 2040(A)

## UNIVERSITY OF DUNDEE

Applications, from candidates who hold or are about to obtain a good Honours degree in Biochemistry or related subject, are invited for appointment as a

### RESEARCH ASSISTANT

to work with Dr P. R. Baker, in the University Department of Surgery at Ninewells Hospital and Medical School, on a study of the effects of cholestasis on cellular transport and protein binding of bile salts by the liver.

The appointment is supported by a S.H.H.D. grant and will be available for three years from September 1, 1979, or some other agreed date. Initial salary at an appropriate point within the range £3,689 to £4,232 (to be reviewed from October 1, 1979). Superannuation under U.S.S. Possibility of registration for a higher degree.

Applications, quoting reference EST/54/79J and containing full details of qualifications and experience and the names of three referees, should be sent to The Secretary, The University, Dundee DD1 4HN by July 5, 1979.

Informal enquiries may be made to Dr P. R. Baker, Department of Surgery, Ninewells Hospital and Medical School, Dundee. 2038(A)

## UNIVERSITY OF SALFORD DEPARTMENT OF CHEMISTRY AND APPLIED CHEMISTRY POSTDOCTORAL RESEARCH FELLOW

required for a project sponsored by the Science Research Council entitled 'SN<sub>2</sub> Reactions Involving Organocuprate Reagents' under the supervision of Dr S. M. Roberts. The appointment is tenable for one year in the first instance, commencing October 1, 1979.

Commencing salary within the range: £3,883 to £4,382 per annum (under review), U.S.S.

Application forms obtainable from the Registrar, University of Salford, Salford M5 4WT (tel: 061-736 5843, ext. 215) to whom completed applications should be returned by not later than June 21, 1979, quoting reference no. CH/258/N. 2003(A)

## university of wales university college of swansea

### Research Demonstrator

Applications are invited for the vacancy of Research Demonstrator in the Department of Botany and Microbiology. Applicants should have a Ph.D. degree acquired by research on some aspect of either Botany or Microbiology. The duties will include demonstrating to practical classes, conduct of tutorials and possibly some lecturing. The successful candidate will be expected to conduct original research and ample time will be left for this.

The appointment, which will be for one year in the first instance from October 1, 1979, will be on a scale up to £4,910 per annum. Further particulars and application forms (2 copies) may be obtained from the Personnel Officer, University College of Swansea, Singleton Park, Swansea SA2 8PP, to whom they should be returned by FRIDAY, JUNE 22, 1979. 2059(A)

**UNIVERSITY OF  
EDINBURGH  
DEPARTMENT OF PHYSICS  
RESEARCH ASSOCIATE/  
FELLOW**

**in Neutron Scattering**

Applications are invited for the post of research associate/fellow to take part in a programme in neutron-scattering experiments associated with the study of structural phase transitions. This project will involve both elastic scattering for high-accuracy, high-resolution studies of crystal structure, and inelastic scattering to make parallel investigations of crystal dynamics. Most experimental work in the immediate future will be at the Institut Laue-Langevin, Grenoble.

The appointment, for up to three years, commences October 1979 (or later by arrangement) at a salary according to age and experience on scale 1A, Superannuation under F.S.S.U./U.S.S. Applicants should have, or be about to obtain, a Ph.D.

Applications with the names of two referees and a statement of applicant's interests and career to date to Dr R. J. Nelmes, Department of Physics, University of Edinburgh, Mayfield Road, Edinburgh EH9 3JZ, to arrive as soon as possible. Further details on request. Please quote Reference 5027. 2011(A)

**NUFFIELD  
LABORATORIES  
OF COMPARATIVE  
MEDICINE  
INSTITUTE OF ZOOLOGY  
ZOOLOGICAL SOCIETY  
OF LONDON**

A recently qualified

**POSTDOCTORAL  
RESEARCH FELLOW**

required for studies on lipid metabolism of the brain with special reference to the role of essential fatty acids and prostaglandins. The successful candidate would be expected to work with a group studying nutrition and the development of the nervous and vascular systems. Experience in lipid biochemistry is essential.

The post will be tenable for 3 years and will carry a salary related to the London University Lecturer scale at a point appropriate to age and experience with U.S.S./J.S.D.P.S. benefits.

Applications in writing to Assistant Establishment Officer, Zoological Society of London, Regents Park, London NW1 4RY. 2053(A)

**UNIVERSITY OF  
SALFORD  
DEPARTMENT OF BIOLOGY  
RESEARCH ASSISTANT  
OR FELLOW**

Applications are invited from biologists/physiologists to assist with a study sponsored by the Agricultural Research Council of respiratory/metabolic physiology in vertebrates. An appointment will be made at the postdoctoral or graduate level depending on qualifications and is tenable for up to 3 years. Informal enquiries concerning the appointment may be made to Dr J. H. Brackenbury, Department of Biology.

Salary within the range: £3,689-£4,776 per annum. U.S.S. benefits.

Application forms are obtainable from the Registrar, University of Salford, Salford M5 4WT (tel: 1-736 5843, ext. 215) to whom completed applications should be returned by June 25, 1979, quoting Ref. No. B/92/N. 2055(A)

**THE RESEARCH  
ORGANISATION OF  
SYNTHELABO**

An Internationally recognised European Pharmaceutical Group located in Paris requires a

**SENIOR TOXICOLOGIST**

to lead a team responsible for various projects within a developing toxicology group in the Department of Biology.

The high success rate and rapid growth of this pharmaceutical group is related to the quality of people they employ and their continued commitment to research and development.

Applicants can be either male or female and should be a Ph.D. with at least five years' postdoctoral experience in toxicology and preferably with industrial experience as well. Suitably qualified veterinarians and pharmacists with relevant experience will also be considered for the post.

Candidates should possess a good working knowledge of French and English and should also be conversant with the G.L.P. regulations for toxicology.

A realistic salary can be expected and relocation assistance will be available together with other financial benefits. All applications will be treated in the strictest confidence and should be sent directly to M R. J. Brault at the following address:

Synthelabo  
58 Rue de la Glaciere  
75013 Paris W151(A)

**THE ROYAL VETERINARY  
COLLEGE**

University of London

Division of Clinical Studies

DEPARTMENT OF

ANIMAL HUSBANDRY

AND HYGIENE

**RESEARCH ASSISTANT**

Applications are invited, preferably from veterinary or animal science graduates, for the above post of Research Assistant, which is expected to be for three years supported by grant from the Ministry of Overseas Development, to take part in a study of nutritional and environmental factors influencing reproduction in ewes and growth of lambs. This is part of a project being undertaken in Colombia and the work will include a laboratory analysis of samples from Colombia.

The post is based at Boltons Park, near Potters Bar, Herts.

Starting salary between £4,191 and £5,278 (including London Allowance) according to qualifications and experience.

Application form from the Assistant Secretary (Personnel), The Royal Veterinary College, Royal College Street, London NW1 0TU. Telephone 01-387 2898. 2041(A)

**New Zealand  
UNIVERSITY OF  
CANTERBURY  
Christchurch**

**VISITING LECTURER  
IN CHEMISTRY**

Applications are invited for a Visiting Lectureship in Chemistry tenable for one year. The appointment is to be taken up as early as possible in 1980. It is anticipated that the appointee will have completed a Ph.D. and show evidence of strong research interest in some particular field of Chemistry.

The emolument for this position is on a scale from NZ\$11,894 to NZ\$14,615 per annum. When the salary is being decided, due account will be taken of the appointee's travel costs.

Applications close on **July 15, 1979**. Further particulars and Conditions of Appointment may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. 1096(A)

**CSIRO AUSTRALIA**

**Postdoctoral Research Fellow  
Australian Numerical Meteorology  
Research Centre  
Melbourne, Victoria**

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees—2,400 of who are research and professional scientists—located in Divisions and Sections throughout Australia.

**Field:** Mathematical Modelling of Climate.

**General:** The Australian Meteorology Research Centre is a joint venture of the CSIRO and the Department of Science (Bureau of Meteorology). The Centre's research consists of studies of the behaviour of the earth's atmosphere, with emphasis on general circulation, directed towards improvement in the accuracy and time scale of weather forecasting, and towards improvement in understanding the distribution and variations of climate on the earth.

In this context the Centre is seeking further support for its climatic activities. Currently the Centre has a number of large scale general circulation models of the earth's atmosphere in use, as well as a more limited modelling involvement in oceanography. The research programme with these models includes detailed simulation of the general circulation, climatic studies, air-sea interaction experiments, investigation of the mechanisms of drought, geophysical experiments and atmospheric chemistry and pollution studies. Additional effort is to be committed to climatic studies using an advanced two level general circulation model developed at the Centre.

**Duties:** Initially to collaborate in experiments with a two level model involving multi-annual integrations with and without coupled oceans with a view to understanding the underlying mechanisms and causes of interannual variability. To formulate appropriate research programmes using this or other models in accordance with the research aims of the Centre.

**Qualifications:** Applicants should have a PhD in dynamical meteorology or an associated field or postgraduate experience of equivalent standard and duration. As considerable initiative is encouraged and expected in the implementation of new programmes, applicants should be highly self-motivated and in particular of high creativity.

**Salary:** Research Scientist \$15,422—\$18,904 pa. or Senior Research Scientist \$19,572—\$22,405 pa. Scientists with considerable experience and outstanding ability should not be deterred from applying as appointment at a higher level and salary would be considered.

**Tenure:** Initially three years with the possibility of an extension of a further two years subject to mutual agreement.

Applications in duplicate, stating full personal and professional details, the names and addresses of at least two professional referees, and quoting reference number 424/029 should reach: The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 6th July, 1979.

Applications in U.S.A. and Canada should be sent to: The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, N.W., Washington, D.C., 20036, U.S.A. 1089(A)

**UNIVERSITY OF ALBERTA**

**FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES**

Applications are invited for a full-time tenurable appointment, effective July 1 or September 1, 1979, in Pharmacaceutics.

The position requires an individual trained at the Ph.D., Pharm.D. level or equivalent and having a special interest and expertise in dosage form design, development and evaluation. Responsibilities include (1) development of an independent research programme in pharmacaceutics, and (2) contribution to the undergraduate and graduate teaching programmes in physical pharmacy and pharmaceutical technology. Appointment will normally be at the rank of assistant professor. Appointment at a higher rank may be considered based upon experience.

The University of Alberta is an equal opportunity employer and offers excellent fringe benefits.

Interested candidates should send a letter of application, a curriculum vitae and the names of three references to:

Dean G. R. Van Petten  
Faculty of Pharmacy and Pharmaceutical Sciences  
Rm. 3118/Dentistry/Pharmacy Centre  
University of Alberta  
Edmonton, Alberta T6G 2N8 W147(A)

## Science Research Council SPACE SCIENCE POST FOR APPLETON LABORATORY/ RUTHERFORD LABORATORY

The SRC requires a person to join its space science team which is concerned with astrophysics and geophysics research and with space project management in support of University programmes. For the first five years of the appointment the selected candidate will be given unpaid leave of absence to enable him/her to take up a post in Oxford University where he/she will be the deputy to the Head of the Department of Atmospheric Physics with particular responsibility for administration and day-to-day organisation of research projects involving satellite, balloon, and aircraft mounted instrumentation. During this period the person appointed will also be expected to pursue research in some aspect of atmospheric physics and to assist with the teaching and supervision of graduate students. At the end of this period the person would be expected to head a team working in some branch of Atmospheric Physics or its management within the Appleton/Rutherford Laboratory at Chilton.

Candidates should have had wide experience of Atmospheric Physics using space research and have had a successful record in major space project management. He/she should be accustomed to working with an academic community.

The post is graded Principal Scientific Officer, with a salary on the scale £6,609—£8,461 (currently under review), with a non-contributory superannuation scheme. Whilst at Oxford University, the successful candidate will be placed on Grade III in the Structure for Research Staff, which has a salary scale of £8,182—£10,097.



Applications, including a full curriculum vitae, should be sent to Mrs A. P. Roythorn, Science Research Council, P.O. Box 18, North Star Avenue, Swindon SN2 1ET, Wiltshire, to arrive before 22nd June, 1979. 2062(A)

## Graduate – Biochemistry

We have a vacancy for an Experimental Officer in the Biochemistry Section of our Chemistry Group. The person we are seeking should have a degree or HNC in Biochemistry or Botany, with a good knowledge of plant biochemistry generally and a sound understanding of photosynthesis specifically. Experience with a range of techniques associated with photosynthesis research and also some experience of enzymology is desirable. He or she will respond to a PhD Biochemist studying biochemical mechanisms involved in herbicide action. This will involve short term research aimed at optimizing chemical structures in *in vitro* tests, as well as longer term research to gain a more precise understanding of the interaction between plants and inhibitors. We offer good salaries, profit sharing scheme and pension fund. A Staff Restaurant and Recreation Club are on site. Subsidised coaches run from Jealott's Hill, Bracknell and Maidenhead. If you are interested in applying for this vacancy please apply to: S R Stephenson, Personnel Officer, ICI Plant Protection Division, Bracknell, Berkshire. 1087(A)



**Plant Protection  
Division**

### UNIVERSITY OF NEWCASTLE UPON TYNE DEPARTMENT OF GENETICS POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited for the above post (salary scale £4,232 to £7,145) from applicants who have or are about to submit for the degree of Ph.D. in any of the following subject areas: Virology, Biochemistry, Genetics, Microbiology or other related field.

The post will be funded from a Wellcome Trust Grant and is tenable for a two-year period (starting date negotiable).

The research project involves characterisation and mapping of peptides obtained from virus proteins using a new analytical procedure. A research technician will assist the successful applicant in this work.

Written applications together with the names and addresses of three referees should be sent to Dr A. C. R. Samson, Department of Genetics, Ridley Building, The University of Newcastle upon Tyne, NEWCASTLE UPON TYNE NE1 7RU. Closing date for applications six weeks after appearance of this advertisement. 2035(A)

### UNIVERSITY OF SURREY DEPARTMENT OF BIOCHEMISTRY LECTURER IN NUTRITION

Applications are invited from suitably qualified graduates for a Lectureship in Nutrition in the Division of Nutrition and Food Science of the Biochemistry Department. Preference will be given to applicants with post-graduate experience in human nutrition.

Salary will be within the range £4,232 to £8,452 per annum depending upon qualifications and experience (£4,333 to £8,992 per annum as from October 1, 1979). Superannuation is under U.S.S. conditions.

Further particulars about the post may be obtained from the Academic Registrar (LFG), University of Surrey, Guildford, Surrey GU2 5XH, or tel: Guildford 71281, ext. 452.

Applications from men or women in the form of a curriculum vitae, together with the names and addresses of two referees, should be sent to the above by June 29, 1979. 1090(A)

### THE UNIVERSITY OF WINNIPEG DEPARTMENT OF BIOLOGY MICROBIOLOGIST

Applications are invited for a sessional appointment at the level of Assistant/Associate Professor to develop and teach courses in Microbial Taxonomy and Immunology and undertake other assigned duties.

The position will be renewable for up to three years. Starting date September 1, 1979. Rank and salary dependent on qualifications and experience. Send curriculum vitae and the names of three referees to: Dr R. A. Woods, Biology Department, The University of Winnipeg, Winnipeg, Manitoba, Canada R3B 2E9. W150(A)

### GLASSHOUSE CROPS RESEARCH INSTITUTE requires SCIENTIFIC OFFICER IN PLANT PHYSIOLOGY DEPARTMENT

for research programme on properties and function of phytochrome. Appointment in Scientific Officer grade. Salary £2,839 to £4,415 (under review). Applicants should preferably have honours degree in Botany, Plant Physiology or Plant Biochemistry, with experience in biochemical techniques. Non-contributory superannuation scheme.

Further details and application form on request from Secretary, G.C.R.I., Worthing Road, Rustington, Littlehampton, W. Sussex BN16 3PU. Closing date: June 20. 2018(A)

### SHEFFIELD CITY POLYTECHNIC DEPARTMENT OF CHEMISTRY CHEMISTRY RESEARCH POSTS

Applications are invited for the following posts to be taken up in 1979. The post holders will be expected to seek registration for M.Phil./Ph.D. degrees of C.N.A.A.

#### POLYMER CHEMISTRY:

##### RESEARCH ASSISTANTSHIP

The successful applicant will work with Dr G. C. Corfield and other members of a Polymer science and technology research group on the synthesis and radiation stability of silicone elastomers. This is a salaried appointment for the fixed term of three years.

Salary: £2,916 to £3,330.

Starting date: September 1, 1979.

#### ANALYTICAL CHEMISTRY:

##### S.R.C. C.A.S.E. STUDENTSHIP

The project concerns trace metal speciation in environmental and other samples by directly coupled chromatography — atomic spectroscopy. The Studentship is in collaboration with Pye-Unicam, Cambridge, and the academic supervisors are Dr L. Ebdon and Dr D. A. Leathard. The S.R.C. award is to be supplemented by £500 per annum. Normal S.R.C. conditions of eligibility apply.

Starting date: October 1, 1979.

Requests for an application form in writing only please to the Recruitment Section of the Personnel Department, Sheffield City Polytechnic (Dept. N), Halfords House, Fitzalan Square, Sheffield S1 2BB. Completed forms should be returned by July 1, 1979. 2013(A)

### UNIVERSITY OF SALFORD DEPARTMENT OF CHEMISTRY AND APPLIED CHEMISTRY POSTDOCTORAL RESEARCH FELLOW

required for work on an S.R.C. sponsored project, 'The Synthesis and Evaluation of Novel Photo-activated Reagents for use in Biological Membrane Studies', under the supervision of Professor H. Suschitzky. The research is suitable for a candidate holding, or expecting to hold a Ph.D. in Organic Chemistry or Biochemistry. Post tenable for up to 2 years.

Salary range: £4,232 to £4,770 per annum. U.S.S. benefits.

Application forms obtainable from the Registrar, University of Salford, Salford M5 4WT (tel 061-736 5843, ext. 215) to whom completed applications should be returned quoting Ref. No CH/259/N. 2056(A)

### UNIVERSITY OF DUBLIN Trinity College LECTURER IN BIOCHEMISTRY

Applications are invited for the above post in the Department of Biochemistry, Trinity College Dublin.

Salary scale: £4,317 to £8,487 per annum.

Appointment may be made within the range commensurate with the qualifications and experience of the successful candidate. There is a non-contributor pension scheme.

Interested persons should, in the first instance, telephone the Staff Office, Trinity College, on Dublin 772941, Ext. 1678 or Ext. 1775.

The closing date for receipt of applications will be Thursday June 28, 1979. W149(A)

**THE UNIVERSITY OF  
LEEDS**  
**DEPARTMENT OF INORGANIC  
AND STRUCTURAL  
CHEMISTRY**

Applications are invited for  
a temporary post of  
**POSTDOCTORAL**

**RESEARCH FELLOW**  
from inorganic or bio-chemists.

Candidates should already possess, or expect to receive within the next few months, a Ph.D. degree. Experience in mechanistic studies would be valuable. The appointee will take part in a project, supported by S.R.C., on electron, supported by S.R.C., on copper proteins, iron-sulphur proteins and cytochrome c. Some collaboration and interchange with Professor R. G. Wilkins in the U.S.A. may be involved. The appointment will be made for a fixed period of up to 2 years commencing October 1, 1979.

Starting salary in the range £4,333 to £4,910 on the 1A scale for Research and Analogous Staff (£4,333 to £7,521) (under review), according to age, qualifications and experience.

Enquiries should be addressed in the first instance to Dr A. G. Sykes, Department of Inorganic and Structural Chemistry, The University, Leeds LS2 9JT (Tel: 0532-31751, ext. 6068/6057).

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 44/7. Closing date for applications: July 2, 1979.

2017(A)

**UNIVERSITY OF GLASGOW**  
**CHEMISTRY DEPARTMENT**  
**GC-MS UNIT**

(Revised Advertisement)

Applications are invited for a  
**POSTDOCTORAL**

**RESEARCH APPOINTMENT**

able for two years from October '81, or investigations on biologically active steroid/cholesterols and related compounds by GC-MS and ancillary techniques. The Unit is equipped with KB 9000 and DuPont 21-490F I/EI instruments, either of which can be used on-line to a VG 2035 data System.

Starting salary in the range £4,333 to £4,910 (the latter for age 26) of the scales for Research and Analogous Staff (1A) with U.S.S. benefits. Further details are available from Professor C. J. W. Brooks, Chemistry Department, University of Glasgow, Glasgow G12 8QQ.

In reply please quote Ref. No. 65M.

2010(A)



**LECTURER  
IN  
BIOCHEMISTRY**

Applications are invited for the above post in the Department of Biochemistry, Trinity College, Dublin.

Salary scale: £4,317 to £8,487 per annum.

Appointment may be made within range £4,317 to £5,492 per annum, a point commensurate with the qualifications and experience of the successful candidate.

There is a non-contributory pension scheme.

Interested persons should, in the first instance, telephone the Staff Office, Trinity College, on Dublin 941, ext. 1678 or ext. 1775.

The closing date for receipt of applications will be **Thursday, June 28, 1979**.

2061(A)

**NATIONAL  
UNIVERSITY OF LESOTHO**  
Applications are invited for the post of  
**SENIOR LECTURER/  
LECTURER**

in Organic, Physical Organic or  
Physical Chemistry

to teach at the General B.Sc. degree level.

Salary scales: Senior Lecturer R7,473 to R8,433 per annum; Lecturer R5,493 to R7,527 per annum. (£1 sterling=R1.72.) The British Government may supplement salary in range £1,644 to £2,508 per annum (sterling) for married appointee or £570 to £1,410 per annum (sterling) for single appointee (reviewed annually and normally free of tax) and provide children's education allowances and holiday visit passages. Superannuation: Appointees on contract terms receive 25% gratuity in lieu of superannuation for the first two years of the contract, rising to 27½% and 30% for each subsequent and similar period of service. 15% inducement allowance for expatriates not qualifying for any supplementation aid scheme. Accommodation is available at reasonable rentals. Family passages; baggage allowance; education allowance; vacation and study leave; medical aid scheme.

Detailed applications (2 copies) with curriculum vitae and naming three referees by July 18, 1979, to the Administrative Assistant (Appointments) NUL, Roma, Lesotho, Africa.

Applicants resident in the U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address.

2000(A)

**UNIVERSITY OF WARWICK**  
**POSTGRADUATE  
RESEARCH ASSOCIATE**

in the

**DEPARTMENT OF  
CHEMISTRY AND  
MOLECULAR SCIENCES**

Applications are invited to the S.R.C. funded post of Postgraduate Research Associate to work with Dr O. W. Howarth on a <sup>13</sup>C nuclear magnetic resonance study of the physical dynamics of biomolecules and macromolecules. Candidates should hold a good honours degree in chemistry or biochemistry. A suitably qualified candidate will be permitted to register for a higher degree on appointment. The post is tenable for three years with a starting salary up to £4,232 per annum on the Research Range 1B scale £3,689 to £5,321 per annum depending on age and experience. Applications, together with the names of two referees, should be received not later than June 29, 1979, by the Academic Registrar, University of Warwick, Coventry CV4 7AL. Please quote Ref. No. 44/A/79.

2042(A)

**QUEEN MARY COLLEGE**  
**University of London**  
**ASTRONOMY—  
APPLIED MATHEMATICS  
POSTDOCTORAL  
RESEARCH ASSISTANT**

required from October 1, to work with Professor I. W. Roxburgh and Dr S. J. Schwartz on an S.R.C. supported project on plasma physics of the solar wind. Applicants should have experience in an area of plasma physics. Appointment for 2 years, with possibility of extension for third year.

Initial salary in range (under review) £4,835 to £5,990 per annum (including London Allowance).

Applications, including age, qualifications, experience and names of 2 referees, should be sent to The Registrar (N), Queen Mary College, Mile End Road, London E1 4NS.

1099(A)

**DIRECTOR AND PROFESSOR  
MEDICAL BACTERIOLOGIST**



**ROBERT KOCH INSTITUTE  
FEDERAL HEALTH OFFICE**

**BERLIN, FEDERAL REPUBLIC OF GERMANY**

The Federal Health Office is a superior Federal authority. Among its terms of reference are practice-oriented research, advisory and executive tasks, preparation of expertise as well as work on medical statistics in the field of Public Health. Its aims are the promotion of consumer protection in respect of health, reduction of health hazards resulting from the environment, detection of causes, early recognition, and control of diseases as well as drug safety. 1,400 people are employed, among them 350 scientists.

Applications are invited for a post involving interesting, responsible and independent work.

**Responsibilities:** As chief bacteriologist, the appointee is responsible for the Enterobacteriaceae Unit which has the status of a National Salmonella and Vibrio Reference Laboratory. He is expected to supervise the staff, to carry on Public Health tasks linked to the diagnostic and epidemiological work of the laboratory and to perform scientific research for the promotion of diagnostic development in the field of enterobacteriaceae with special reference to enterotoxinogenic germs.

**Qualifications:** M.D. or equivalent in microbiology, clinical bacteriologist preferred. Several years' experience in bacteriology and/or diagnosis of bacterial infections with special reference to enteropathogenic bacteria. The candidate is expected to present a list of scientific publications on relevant subjects.

**Languages:** Excellent knowledge of English or good working knowledge of other languages such as French or German.

**Initial salary:** 60,000 DM/year. The Office will assist the successful applicant in the procurement of housing. Allowance for temporary separation from family is granted under applicable regulations.

Applications with curriculum vitae, photograph, brief outline of professional career, copies of credentials, and a list of publications should be sent by July 16, 1979, to the

**PRESIDENT OF THE FEDERAL HEALTH OFFICE**  
**Postfach: D-1000 Berlin 33**

quoting reference number 367 01.

For further information please call: (030) 8308 569 (Herr Pankonin).

W145(A)

**ULSTER POLYTECHNIC**  
**RESEARCH OFFICER**

(TWO-YEAR APPOINTMENT)

Salary Scale: £3,732 to £4,632 (under review)

A Research Officer is required to work in the Chemistry Division of the School of Physical Science on one of the following projects:

- Electrochemical fixation of carbon dioxide;
- Synthesis and testing of fungicides;
- Investigation of the Starch enzymology of Bacillus Macerans.

Applicants must have a good Honours degree or equivalent in Chemistry. A higher degree with appropriate research experience will be an added advantage.

The Polytechnic is a direct grant institution with an independent Board of Governors. It opened in 1971 and has a student population of some 7,500. It has extensive new purpose-built accommodation, including 750 residential places on the 114-acre campus overlooking the sea at Jordanstown, a pleasant and quiet residential area. There is a scheme of assistance with removal.

Further particulars and application forms which must be returned by June 25, may be obtained by telephoning Whiteabbey (0231) 65131, ext. 2243 or by writing to:

The Establishment Officer, Ulster Polytechnic, Shore Road, NEWTOWNABBEY, Co. Antrim BT37 0QB.

2025(A)



## CSIRO AUSTRALIA

### Postdoctoral Research Fellow

#### Division of Forest Research Queensland Regional Station Atherton, Queensland

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees—2,400 of who are research and professional scientists—located in Divisions and Sections throughout Australia.

**Field:** Forest Soils Research.

**General:** The Division has a small group located at Atherton in North Queensland undertaking research in aspects of rainforest botany and ecology. Atherton lies within the main belt of tropical rainforest in northeastern Australia and is well situated as a base for field research with a wide variety of rainforest types in the immediate vicinity.

**Duties:** To initiate and undertake research relating to the influence of soil properties on the distribution of rainforest plant species and the effects of various forest management alternatives on the stability of rainforest soils. It is envisaged that the work will be carried out in co-operation with other members of the Station and soil scientists from the Division of Soils located in Townsville.

**Qualifications:** A Ph.D. or equivalent qualification in a relevant field, preferably soil physics.

**Salary:** Research Scientist/Senior Research Scientist \$15,422—\$22,405 p.a.

**Tenure:** 5 years superannuation available.

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 1050/131 should reach:—

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 6th JULY, 1979.

Applications in U.S.A. and Canada should be sent to:—  
The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, N.W., Washington, D.C., 20036, U.S.A.

1088(A)

### M.R.C. CLINICAL RESEARCH CENTRE (Northwick Park Hospital) Watford Road, Harrow, Middlesex HA1 3UJ

We require a

#### Ph.D. PHARMACOLOGIST

with experience in experimental cancer chemotherapy or in evaluating toxicity of antitumour drugs in experimental cancer chemotherapy. This work will be carried out under a grant awarded for two years by the National Institute of Health. This appointment will therefore be for two years on a salary scale starting from £3,883 to £6,080 per annum plus £502 London Allowance.

Application form obtainable from Mrs J. Tucker-Bull, 01-864 5311, ext. 2685. Please quote Ref. 111/1/NIH/SC. Closing date: June 22, 2008(A)

### THE ANIMAL VIRUS RESEARCH INSTITUTE Pirbright, Woking Surrey GU24 0NF MICROBIOLOGIST/ IMMUNOLOGIST

Applications are invited for a microbiologist/immunologist to work on the antigen and antibody relationship in foot-and-mouth disease and possibly other viruses which show marked antigenic variation. Recent work has established the possibility of production of monoclonal antibodies and it is proposed to intensify work on this topic. Candidates should have a good honours degree and at least four years' postgraduate experience in immunology or virology.

The post is in the S.S.O. grade £5,154 to £6,898, under review). House available for suitable married applicant. Non-contributory superannuation scheme.

Application forms and further particulars from the Secretary.

2028(A)

### UNIVERSITY OF SOUTHAMPTON

#### MEDICAL ONCOLOGY UNIT

Applications are invited for the position of Postdoctoral Research Fellow in the above Unit. The person appointed will work on the isolation of cytoskeletal proteins and their interaction with the cell surface, in close cooperation with a group interested in cell adhesion and with others interested in clinical aspects of cancer research. Applicants should either be about to obtain or have recently obtained a Ph.D. in Biochemistry or some related discipline. Experience of protein biochemistry, or immunology would be advantageous. The appointment will be for three years.

Salary Range: £4,333 to £5,488 per annum. U.S.S. benefits.

Applications giving date of birth, curriculum vitae and the names and addresses of two referees, should be sent to Mrs P. Vaughan-Smith, The University, Southampton SO9 5NH, not later than June 22, 1979. Please quote reference 1069/R. 2044(A)

### International Laboratory for Research on Animal Diseases I.L.R.A.D.—Nairobi—Kenya RESEARCH ASSISTANT

To work on the Immunology and Parasitic diseases. There are three vacancies for research assistants in the laboratory of the Director of I.L.R.A.D. The research assistants should be qualified and should have experience on tissue culture, immunology or parasitic diseases.

#### TERMS:

The salary will depend on age and experience. Perquisites include moving allowance, housing and commutation allowances, medical and retirement benefits. Applications, with curriculum vitae, and the names of three referees should be submitted to

The Director

I.L.R.A.D.

P.O. Box 30709

NAIROBI—KENYA

To reach him not later than June 28, 1979. W137(A)

### UNIVERSITY OF CAMBRIDGE BIOMETRY

vacant

### UNIVERSITY DEMONSTRATORSHIP in the DEPARTMENT OF APPLIED BIOLOGY

to support teaching and research in Biometry with special emphasis on use of the University computer. Good Honours degree in Mathematics, Statistics or Computer Science and/or postgraduate experience of biometrical analysis and computer-programming.

Salary in scale £4,505 to £5,591, pensionable. Limited contribution to removal expenses.

Further details from and applications including full personal particulars, list of publications and names and addresses of not more than three referees to the Secretary, Faculty of Biology 'A' Appointments Committee, Department of Applied Biology, Pembroke Street, Cambridge CB2 3DX, not later than July 7, 1979. 2064(A)

### CENTRAL MIDDLESEX HOSPITAL DEPARTMENT OF GASTROENTEROLOGY RESEARCH ASSISTANT

A Research Assistant is required work on a Research Programme concerned with clinical nutrition. The work is concerned with investigating the pathophysiology of diarrhoea that occurs during Enteral Nutrition.

The post is for two years at an initial salary of £3,689 (plus £354 London Allowance). It is hoped that the appointee will register for a higher degree.

Applications and enquiries should be sent to Dr D. B. A. Silk, Department of Gastroenterology, Central Middlesex Hospital, London NW10 not later than July 1, 1979.

2048(A)

### UNIVERSITY OF LIVERPOOL ORTHOPAEDIC AND ACCIDENT SURGERY TECHNICIAN

for research project concerning the biological approach to replacement of arthritic joints. Facilities are available within the department for cartilage and bone cell culture. Previous experience of immunological techniques desirable, but training given. Candidates must possess H.N.C. or an appropriate equivalent qualification and have had several years laboratory experience. Salary within a range up to £4,056 per annum, according to qualifications and experience.

Application forms available from the Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote Ref. RV/628/N. 2046(A)

### PRESTON POLYTECHNIC

Applications are invited for the post of

### PRINCIPAL LECTURER IN ASTRONOMY AND DIRECTOR OF THE POLYTECHNIC OBSERVATORIES

Salary: Principal Lecturer £7,047 to £7,818 (bar) to £8,844 under review.

Further particulars and application forms are available from the Personnel Officer, Preston Polytechnic, Corporation Street, Preston PR1 2TQ, to whom completed applications should be returned within 14 days of this advertisement. 2034(A)

### UNIVERSITY OF MASSACHUSETTS

Three positions in protein bio synthesis available immediately in the Department of Biochemistry.

1. POSTDOCTORAL to work on elucidating the topography of ribosomal binding sites for initiation factors and tRNA by photochemical labelling.

2. POSTDOCTORAL to work on the analysis of ribosome assembly reactions by photoaffinity and fluorescence techniques.

3. ASSISTANT PROFESSOR RESEARCH ASSOCIATE to teach course in molecular biology and to participate in research on ribosome structure and function, with emphasis on the physicochemical characterisation of protein-nucleic acid interactions.

Experience in protein synthesis, nucleic acid chemistry desirable. Appointments of one to three year can be arranged. Send resume, in including the names of three reference and position applied for, to Dr I Schwartz or Dr R. A. Zimmermann Department of Biochemistry, University of Massachusetts, Amherst Massachusetts 01003, U.S.A.

An Affirmative Action/Equal Opportunity Employer. W153(A)

### LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

(University of London)  
Keppel Street WC1E 7HT

#### DEPARTMENT OF HUMAN NUTRITION

CLINICAL NUTRITION AND  
METABOLISM UNIT

ST. PANCRAS HOSPITAL

#### A POSTDOCTORAL RESEARCH FELLOW

is required in September 1979 to work for up to two years on a project sponsored by the Medical Research Council for studies on the regulation of protein synthesis and breakdown in animal tissues.

The salary is in the range £4,360 to £7,145 (under review) plus £50 London Weighting.

Applications, consisting of curriculum vitae and naming two referees, should be addressed to Professor J. C. Waterlow at the C.N.M.U. 4 St. Pancras Way, London NW1. 2066(A)

### KINGSTON POLYTECHNIC RESEARCH OPPORTUNITIES (Polymer Research) SCHOOL OF CHEMICAL AND PHYSICAL SCIENCES

Two posts of Research Assistant are available, one at a more senior level (starting salary £3,500) than the other (starting salary £2,940), for three-year studies of the behaviour of crosslinked polymers and related materials in host wet environments. Good honours graduates should contact the Academic Registry, Dept. AO, quoting reference NTL for details and application forms (to be returned as soon as possible) Kingston Polytechnic, Penrhyn Road Kingston upon Thames KT1 2EE. Tel. 01-549 1366. 2014(A)

# INTERNATIONAL CROPS RESEARCH INSTITUTE FOR THE SEMI-ARID TROPICS (I.C.R.I.S.A.T.)

Hyderabad, India

needs the following personnel to work in West Africa.

## PLANT BREEDER (PEARL MILLET)

Responsible for planning and conducting research, in cooperation with a team of international scientists, to develop varieties and advanced breeding materials. Qualifications: Ph.D. in Plant Breeding with excellent academic record; at least five years' research experience in plant breeding, preferably of millet as evidenced by publications/reports, and in organisation and coordination of research. Knowledge of modern techniques in crop improvement and experience of semi-arid tropics would be advantageous. Bilingual ability in English and French preferred.

## SOIL AND WATER ENGINEER

Responsible for collection, analysis and interpretation of research information in management of natural resources and for evaluation of representative farming systems existing in the region. Qualifications: Doctorate with an excellent academic record in Soil and Water Engineering, Soil Physics, or Agricultural Engineering, with substantial formal training in hydrology; proven ability for teamwork with other scientists in interdisciplinary research programmes. Experience in agricultural research pertaining to less developed countries of the S.A.T., at least five years' postdoctoral research experience, and ability to speak and write French would be advantageous.

## AGRONOMIST

Responsible for devising and executing research programmes on cropping systems, soil fertility and soil management for pearl millet, groundnut, and other crops in the region, in consultation with a team of international scientists. The research will be aimed at developing systems of farming which increase and stabilise yields. Qualifications: Ph.D. in Agronomy or associated science; seven years' experience in agronomic research ability to lead a team of scientists in interdisciplinary research. Experience in agricultural research in less developed countries of the S.A.T., knowledge of I.C.R.I.S.A.T.'s Farming Systems Research, and fluency in French would be desirable.

Salary commensurate with qualifications; excellent fringe benefits.

Send resume by September 15, 1979 to: Director, I.C.R.I.S.A.T., 1-11-256, Begumpet, Hyderabad 500 016, India. W152(A)

# SOUTH GLAMORGAN HEALTH AUTHORITY (TEACHING)

## UNIVERSITY HOSPITAL OF WALES

Department of Haematology

## SCIENTIFIC OFFICER

There is a vacancy for a graduate in Mathematics to work as a Scientific Officer in the Department of Haematology in the University Hospital of Wales. This post would be suitable for a person wishing to apply their mathematical expertise to medicine within an active clinical and laboratory environment. In addition to routine duties relating to data processing and quality control, the successful applicant would be expected to take part in the research and development activities of the Department. The development of new mathematical applications within the field of Haematology would be encouraged.

The appointment will be at Scientific Officer level within the scale of £3,486 to £4,899 (under review)—Whitley Council Terms and Conditions of Service.

Further particulars may be obtained from:

Dr I. Cavill. Tel. (0222) 759444, Ext. 2365/2364.

Application forms from: Personnel Department, University Hospital of Wales, Heath Park, Cardiff. Tel. (0222) 755944, Ext. 2917.

Closing Date: June 22, 1979.

2047(A)

# M.R.C. CLINICAL RESEARCH CENTRE (Northwick Park Hospital)

Watford Road, Harrow, Middlesex HA1 3UJ

DIVISION OF PERINATAL MEDICINE

## POSTDOCTORAL ENZYMOLOGIST

required to work on developmental enzymology in man with emphasis on mitochondrial systems. Previous experience in the biochemistry of mitochondria is therefore desirable. Further details are available from Dr R. A. Harkness, Division of Perinatal Medicine, Clinical Research Centre, Harrow HA1 3UJ, Middlesex. Tel: 01-864 5311, ext. 2712.

This appointment will be for three years only. Salary within the range £4,408 to £6,080 plus £502 London Allowance. Further details and application form from Mrs J. Tucker-Bull, quoting Reference 133/1/4267. Closing date: July 2. 2007(A)

# UNIVERSITY OF GLASGOW POSTDOCTORAL RESEARCH ASSISTANTSHIP DEPARTMENT OF BIOCHEMISTRY

Applications are invited for an M.R.C. funded Research Assistantship to be filled at postdoctoral level. The post is with a group studying the molecular biology of antibody formation with emphasis on the application of recombinant DNA technology. Experience in molecular immunology or recombinant DNA techniques is desirable, but a general background in nucleic acid biochemistry would be suitable.

Containment facilities (categories 1, 2 and 3) are available in the Department of Biochemistry and cloning of murine and human genes is in progress.

The position is available from July 1, 1979, and is funded for 5 years.

Salary will be within Range 1A of the salary scales for Research and Analogous Staff (£4,232 to £7,145, under review) with appointment at a point appropriate to age and experience.

Application with curriculum vitae and names of two referees should be sent as soon as possible to Professor R. A. Williamson, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ.

In reply please quote Ref. No. 4464M. 2009(P)

# UNIVERSITY OF LIVERPOOL

DEPARTMENT OF  
BIOCHEMISTRY

## Applications are invited from ORGANIC CHEMISTS/ BIOCHEMISTS

for the post of Postdoctoral Senior Research Assistant to join a group working on the biosynthesis and metabolism of the ecdysteroids in insects and plants. Experience of steroid or terpenoid chemistry will be an advantage but not essential.

The appointment is tenable for a period up to two years and would start as soon as possible but preferably not later than October, 1979. Initial salary within the range £4,232 to £5,048 per annum (under review) plus U.S.S. Benefits.

Applications, together with the names of two referees, should be received as soon as possible by The Registrar, The University, P.O. Box 47, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/629/N. 2045(A)

# ASSISTANTSHIPS

## BRIGHTON POLYTECHNIC

FACULTY OF NATURAL AND  
LIFE SCIENCES

## RESEARCH ASSISTANTSHIPS

Vacancies exist in the following Departments:

APPLIED CHEMISTRY  
APPLIED PHYSICS  
PHARMACY

Salary scale: £2,613 by £87(2) to £2,787.

Details of posts and application forms from Administrative Officer, Faculty of Natural and Life Sciences, Brighton Polytechnic, Moulsecoomb, BRIGHTON BN2 4GJ, to whom completed forms should be returned by Friday, July 13. 2068(A)

# ASSISTANTSHIPS—cont.

## UNIVERSITY OF NEWCASTLE UPON TYNE DEPARTMENT OF BIOCHEMISTRY JUNIOR

## RESEARCH ASSOCIATE

Applications are invited from Honours Graduates for the position of Junior Research Associate (Salary Scale 1B: £3,689 to £5,321) in the above department. This position, which is funded by a project grant from the Medical Research Council, is available from October 1, 1979, for a period of three years. It may be possible for the successful applicant to register for the degree of Ph.D.

The work will involve a study of the regulation of the hormone-sensitive triglyceride lipase from adipose tissue, and will utilise a wide variety of techniques of enzymology and protein chemistry.

Applications, including a curriculum vitae and the names of two referees, should be sent as soon as possible to Dr S. J. Yeaman, Department of Biochemistry, Ridley Building, University of Newcastle upon Tyne NE1 7RU. 2052(A)

## KINGSTON POLYTECHNIC SCHOOL OF GEOLOGY RESEARCH ASSISTANTSHIP

Research Assistant required from August 1979 to work on

THE BIOSTRATIGRAPHY AND  
GEOCHEMISTRY OF  
PHOSPHATE-RICH STRATA IN  
EUROPE AND NORTH AFRICA

Directed jointly by Drs R. T. J. Moody and I. F. Hazell. The selected candidate will be expected to register for a higher degree.

Salary range £2,940 to £3,114 inclusive.

Details and application forms (to be returned by June 29, 1979) quoting Ref. NT3, from Academic Registry, Dept. AO, Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. Tel: 01-549 1366. 2015(P)

# AWARDS

## UNIVERSITY OF NOTTINGHAM DEPARTMENT OF ZOOLOGY

Applications are invited for a

## C.A.S.E. AWARD

to work on the mechanisms of uptake of tickicides by the cattle tick (*Boophilus* sp.). Candidates should have a good honours degree in zoology and a special interest in physiology and cytology. The work which can lead to a higher degree will be carried out in the Zoology Department, Nottingham University and Boots Animal Research Centre, Thurgarton.

Applications should be addressed to the Department of Zoology, Nottingham University, closing date June 18, 1979. 2022(N)

# STUDENTSHIPS and FELLOWSHIPS

## GUY'S HOSPITAL MEDICAL SCHOOL (University of London) PHYSICS DEPARTMENT

## AN M.R.C. STUDENTSHIP

is offered for work leading to a Ph.D. in: the Non-Invasive Investigation of Cardiac/Vascular Disease using Doppler-Shift Ultrasound.

Candidates should possess or expect to achieve a 1st or 2nd Class Honours degree in Physics or a Life Science.

Apply in writing, with a brief curriculum vitae and the names of two referees, to the Secretary, Guy's Hospital Medical School, London Bridge SE1 9RT, by June 15, 1979. 2006(F)

Continued on next page

## STUDENTSHIPS and FELLOWSHIPS continued

**UNIVERSITY OF EDINBURGH**  
**DEPARTMENT OF CHEMISTRY**
**S.R.C. C.A.S.E.****RESEARCH STUDENTSHIPS 1979**

Applications are invited for S.R.C. C.A.S.E. Research Studentships starting in October 1979 on the topics and with supervisors and companies listed below:

- Dipolar Cyclo Additions in Polymerisation  
Dr R. M. Paton/I.C.I. Organic Division
- Vinylchloride Precipitation Polymerisation  
Dr W. D. Cooper/I.C.I. Plastics Division
- Micro-columns for HPLC  
Professor J. H. Knox/I.C.I. Organics Division
- Catalytic Reactions of Hydrocarbons on Treated Aluminas  
Professor C. Kemball/I.C.I. Petrochemicals Division
- Mechanism of Dissolution of Organic Solutes by Water  
Dr B. M. Lowe/Unilever Research Ltd.

Applicants should hold or expect to hold at least an upper second class honours degree in chemistry or a related subject and should write, giving the names of two academic referees, as soon as possible to:

**Professor C. Kemball,**  
**Department of Chemistry,**  
**University of Edinburgh,**  
**West Mains Road,**  
**EDINBURGH EH9 3JJ**

2054(F)

**UNIVERSITY**  
**OF YORK**
**DEPARTMENT OF BIOLOGY**  
**S.R.C. C.A.S.E.**  
**STUDENTSHIP**

with Dr M. Davies, for three years from October 1, 1979, in collaboration with Simon-Hartley Ltd., Stoke-on-Trent. Enhanced efficiency in waste-water treatment is sought through studies of the physiology and biochemistry of growth in a novel completely mixed microbial film fermenter. The dependence of the population distribution on oxygen and substrate gradients within the biomass support particles is of fundamental interest.

Candidates should have or expect to obtain a first or upper second class honours degree in microbiology or biochemistry.

Apply to Dr M. Davies, Department of Biology, University of York, York YO1 5DD (telephone 0904 59861, ext. 5833), from whom further information may be obtained. 1091(F)

**UNIVERSITY OF**  
**SOUTHAMPTON**  
**DEPARTMENT OF**  
**ELECTRICAL ENGINEERING**  
**RESEARCH IN**  
**ELECTRICAL MACHINES**  
**TWO C.A.S.E.**  
**STUDENTSHIPS**

are available from October 1979. One of these is sponsored by G.E.C. Turbine Generators Limited, Stafford, and is concerned with magnetic field problems in large generators. The other is sponsored by the Central Electricity Research Laboratories, Leatherhead, and is concerned with electromagnetic problems in superconducting generators. Several weeks each year will be spent with these sponsors who will augment the basic S.R.C. grant by at least £550 plus expenses.

The successful candidates will be expected to register for a higher degree.

Applications, with curriculum vitae and the names of two referees to Mrs M. Campbell, Department of Electrical Engineering, The University, Southampton SO9 5NH. Please quote Ref. N. 2058(F)

**Royal Holloway College**  
**(University of London)**
**S.R.C. C.A.S.E. Studentships**

Applications are invited for two S.R.C. C.A.S.E. Studentships in the Department of Chemistry.

- (1) "C I mass spectrometry of synthetic base oils", with Dr R A Hancock and Burmah Castrol Company.
- (2) "Computer simulation of the interface between ionic crystals and solution", with Professor K Singer and Unilever Research Laboratory.

Applications, giving the names of two referees should be sent as soon as possible to the Registrar, (N) Royal Holloway College, Egham, Surrey, TW20 0EX. Further particulars may be obtained from the Department of Chemistry (Tel. Egham 5351). 2039(F)

**Athrofa Gogledd-dd Cymru**  
**The North E. Wales Institute**  
**of higher education**

**KELSTERTON COLLEGE, CONNAH'S QUAY,**  
**DEESIDE, CLWYD**

**S.R.C. C.A.S.E.**  
**STUDENTSHIPS**

Applications are invited for two S.R.C. C.A.S.E. Studentships, tenable from 1st October, 1979 for work on the following projects:

1. "Mechanism of lipid oxidation in aqueous emulsions", in collaboration with Unigate Ltd. Supervisor: Dr J. C. Allen.
2. "Radioimmunoassay of polyamines", in collaboration with Ortho Diagnostics, Ltd. Supervisors: Drs J. C. Allen and C. J. Smith.

The co-operating bodies will supplement the SRC Studentships by £250 p.a. Applicants should possess or expect to obtain 1st or upper 2nd class Honours, and should write as soon as possible, with curriculum vitae, to the Institute Registrar, North East Wales Institute, Kelsterton College, Connah's Quay, Deeside, Clwyd CH5 4BR.

1061(F)

**UNIVERSITY OF YORK**  
**DEPARTMENT OF**  
**BIOLOGY**  
**S.R.C. C.A.S.E.**  
**STUDENTSHIP**

An S.R.C. C.A.S.E. Studentship is available for three years from October 1979 to work under the supervision of Dr A. W. Robards in collaboration with I.C.I. Plant Protection Ltd. (Jealott's Hill) on the effects of formulation adjuvants on plant cuticles. The work will involve a range of radiochemical, physiological and electron microscopical techniques with the aim of improving the uptake of foliar applied pesticides.

Candidates should have, or expect to obtain, a first or upper second class honours degree in a relevant biological subject.

Further information may be obtained from Dr A. W. Robards, Department of Biology, University of York, York YO1 5DD (tel. 0904 59861, ext. 5822).

2043(F)

**UNIVERSITY OF**  
**READING**  
**S.R.C. RESEARCH**  
**STUDENTSHIP**

Applications are invited from British or Commonwealth subjects for an S.R.C. Research Studentship tenable for 3 years in the Department of Microbiology from October 1, 1979. The successful applicant, who must have at least a Class 2 division 1 honours degree in Microbiology, Biochemistry or a related subject, will do research for a higher degree. Projects are available in microbial physiology and biochemistry, bacteriology, virology, serology, ecology and genetics and applicants should indicate their area of interest when applying.

The value of the award is £1,610 per annum tax-free (under review) with remission of approved fees.

Application form from Dr L. J. Zatman, Department of Microbiology, University of Reading, London Road, Reading RG1 5AQ. (Ref. M. 38A.) 2004(F)

**UNIVERSITY OF ABERDEEN**  
**DEPARTMENT OF**  
**OBSTETRICS AND GYNAECOLOGY**  
**M.R.C. POSTDOCTORAL**  
**RESEARCH FELLOWSHIP**

Applications are invited from suitably qualified biochemists to work as part of a clinical group, under Professor Klopfer, on isolation, assay and clinical application of new proteins isolated from the placenta.

Experience in immunological techniques and protein handling would be advantageous.

Appointment will be for 2 years, \* commence as soon as possible. Salary within Range 1A, £4,232 to £7,140 per annum (under review), with appropriate placing.

Further particulars from The Secretary, The University, Aberdeen with whom applications (2 copies) should be lodged by June 29, 1979. 2016(E)

**GUY'S HOSPITAL**  
**MEDICAL SCHOOL**  
**(University of London)**
**BIOCHEMISTRY AND**  
**CHEMISTRY DEPARTMENT**

Applications are invited for a

**RESEARCH STUDENTSHIP**

in Biochemistry, funded by the Muscular Dystrophy Group of Great Britain, to work with Dr D. C. Wat over three years, on biochemical changes in the human erythrocyte membrane in relation to neuromuscular disease.

Applicants should have a good honours degree and will be expected to register for a Ph.D.

Apply in writing, with curriculum vitae and the names of two referees to the Secretary, Guy's Hospital Medical School, London Bridge Street 9RT, quoting Ref. B.C.2. 2020(F)

**UNIVERSITY OF ABERDEEN**  
**DEPARTMENT OF BOTANY**

Applications are invited for S.R.C. C.A.S.E. Studentship for three years in Plant Pathology to work in the above department and at the Scottish Horticultural Research Institute, Dundee, commencing October 1, 1979. The project is to develop inoculation technique for *Elsino veneta* (a fungal pathogen of raspberry).

Application forms and further particulars from the Science Faculty Office, University of Aberdeen, Regent Walk, Old Aberdeen. (2031)J

**UNIVERSITY OF  
WARWICK  
RESEARCH FELLOWSHIP  
IN VIROLOGY**

Applications are invited for a Post-doctoral Fellowship in the Tumour Virus Research Group in the Department of Biological Sciences. The successful applicant will work in a small group studying the biochemistry of C type RNA tumour viruses, with particular reference to their ability to transform cells. Experience of nucleic acid technology and in particular the use of *in situ* hybridization techniques, while not essential, would be an advantage. Starting salary will be between points 1 and 6 on the Research 1A scale: £4,232 (£5,591) £7,145 per annum. The Fellowship (funded by the Medical Research Council) is available from October 1, 1979 and is renewable on an annual basis until September 1981. Further details and application forms from the Academic Registrar, University of Warwick, Coventry, CV4 7AL quoting Ref. No. 43/A/79. Closing date for receipt of applications is June 22, 1979. (2036)E

**UNIVERSITY OF  
EDINBURGH  
DEPARTMENT OF  
BIOCHEMISTRY  
POSTDOCTORAL  
RESEARCH FELLOWSHIP**

Applications are invited for the above post supported by the Medical Research Council and tenable for 18 months from October 1, 1979. The project will investigate the biological significance of cytochrome c methylation and the use of methylation as a means of studying protein: protein interactions. Some experience in protein chemistry or enzyme purification is desirable.

Starting salary in the range £4,232 to £5,048 per annum.

Further information may be obtained from Dr G. W. Pettigrew, Veterinary Unit, Department of Biochemistry, Royal (Dick) School of Veterinary Studies, Summerhall, Edinburgh EH9 1QH, to whom applications including the names of three referees should be sent before June 30, 1979. Please quote Reference 5067. 2012(E)

**THE UNIVERSITY OF  
MANCHESTER  
S.R.C. C.A.S.E.  
STUDENTSHIP**

Applications are invited for a three-year C.A.S.E. Studentship, starting October 1, 1979, in the Organic Section of the Department of Chemistry for research leading to the degrees of M.Sc. and Ph.D. under the supervision of Dr J. M. Bruce in collaboration with Dr J. Elks of Glaxo Research Ltd., Greenford, on the synthesis of anthracyclines and related compounds. Applicants should hold, or expect to hold, at least an upper second class honours degree in chemistry, or its equivalent.

Further details can be obtained from Dr J. M. Bruce, Department of Chemistry, The University, Manchester M13 9PL, to whom applications, with curriculum vitae and the names of two academic referees, should be sent as soon as possible. 1095(F)

**UNIVERSITY OF OXFORD  
PHYSICAL CHEMISTRY  
LABORATORY**

**Applications are invited for a  
C.A.S.E. STUDENTSHIP**

in collaboration with the Meteorological Office, Bracknell, for the study of atmospherically important energy transfer processes at low temperatures using pulsed laser techniques. The studentship is available from October 1979, and candidates should have, or expect to receive, a First or Upper Second Class Honours degree in Chemistry, Chemical Physics, or a related subject.

Applications, which should include details of academic experience, and the names of two referees, should be made as soon as possible to Dr C. J. S. M. Simpson, Physical Chemistry Laboratory, South Parks Road, Oxford OX1 3QZ. 2063(F)

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SANDOZ Forschungsinstitut,  
A-1235 Wien, Brunner Straße 59,  
Austria.

(W 146)E

**UNIVERSITY OF  
CAMBRIDGE  
DEPARTMENT OF  
PHARMACOLOGY  
M.R.C.**

**RESEARCH STUDENTSHIP**

Applications are invited for a 3-year studentship to commence in October 1979. Candidates should have, or expect to obtain this year, a good honours degree in Biochemistry, Biophysics, Pharmacology, Physiology, or related subjects. The successful candidate will be expected to register for a Ph.D. and pursue research into pharmacological aspects of insulin secretion and the molecular basis of hypoglycaemic drug action. The research methodology involved will be multidisciplinary.

Applications together with a curriculum vitae and the names and addresses of two referees should be sent as soon as possible to Dr E. K. Matthews, University Reader in Pharmacology, Department of Pharmacology, University of Cambridge, Hills Road, Cambridge CB2 2QD. 2029(F)

**M.R.C.  
RESEARCH STUDENTSHIP  
DEPARTMENT OF  
HUMAN NUTRITION  
LONDON SCHOOL OF  
HYGIENE AND TROPICAL  
MEDICINE**

(University of London)

Applications are invited for a Research Studentship to join a group investigating the regulation of growth, protein synthesis and degradation in muscle. The work is taking place at the Clinical Nutrition and Metabolism Unit at St. Pancras Hospital.

Applicants should expect to gain a first or second class honours degree in Biochemistry, Physiology, or Nutrition, and should be able to start in October, 1979.

Applicants should send a curriculum vitae together with the names of two referees to Dr D. J. Millward, Clinical Nutrition and Metabolism Unit, 4 St. Pancras Way, London NW1, as soon as possible. 2057(F)

**THE UNIVERSITY OF  
SHEFFIELD  
Wolfson Unit of Plant Cell  
Biotechnology  
DEPARTMENT OF  
BIOCHEMISTRY  
S.R.C. C.A.S.E.  
STUDENTSHIP**

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**UNIVERSITY OF SUSSEX  
SCHOOL OF  
BIOLOGICAL SCIENCES  
RESEARCH COUNCIL  
STUDENTSHIPS  
IN DEVELOPMENTAL  
GENETICS**

Applications are invited for post-graduate studentships (to commence October 1979) for research on

- The genetics of early embryonic determination and imaginal disc morphogenesis in *Drosophila melanogaster* (with Dr J. R. S. Whittle), and
- Early detection of genetic and developmental lesions induced by transplacental exposure to toxic chemicals (with Professor R. J. Cole).

Applicants should write as soon as possible to either of the above at School of Biological Sciences, The University of Sussex, Falmer, Brighton, Sussex BN1 9QG. 2051(F)

**UNIVERSITY OF  
READING  
DEPARTMENT OF  
APPLIED STATISTICS  
S.R.C. RESEARCH  
STUDENTSHIPS**

Applications are invited: one is an S.R.C. C.A.S.E. Studentship, in collaboration with A.R.C. Letcombe Laboratory near Wantage, concerned with the development and testing of mathematical models of cell proliferation in relation to plant growth; the other is a straight S.R.C. Studentship and involves applications of probability or statistics in some agreed area.

Apply, quoting Ref. M. 39A, with curriculum vitae and the names of two referees to Professor R. N. Curnow, Department of Applied Statistics, University of Reading, Whiteknights, Reading RG6 2AH, from whom further particulars are available. 2005(F)

**UNIVERSITY COLLEGE  
CARDIFF  
DEPARTMENT OF GEOLOGY  
S.R.C. (C.A.S.E.)  
STUDENTSHIP**

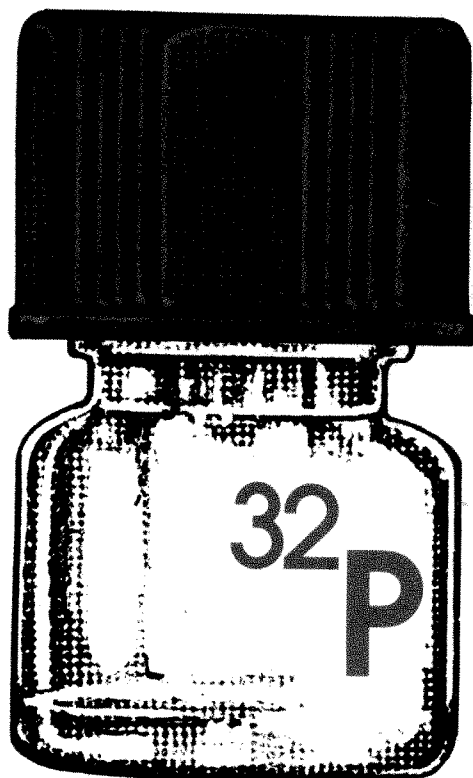
for research, leading to the degree of Ph.D., into the development of a new analogue video comparison and measurement system for a wide variety of shape-analysis applications in the natural, physical and applied sciences, and in industry. The project is run jointly by the Geology Department and E.O.S. Electronics Ltd.

Applicants should possess or expect to gain a first or upper second class Honours Degree in electronics or a closely related subject. Experience in a natural science would be an advantage.

Applications should be made as soon as possible to the Head of Geology Department, University College, P.O. Box 78, Cardiff CF1 1XL, from whom further details may be obtained. 2049(F)







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\*Method of Maxam, A.M., and Gilbert, W., *Proc. Nat'l Acad. Sci. USA*, 74, 560-564 (1977).

\*\*Method of Rigby, P.W.J., Dieckmann, M., Rhodes, C., and Berg, P., *J. Mol. Biol.*, 113, 237-251 (1977).



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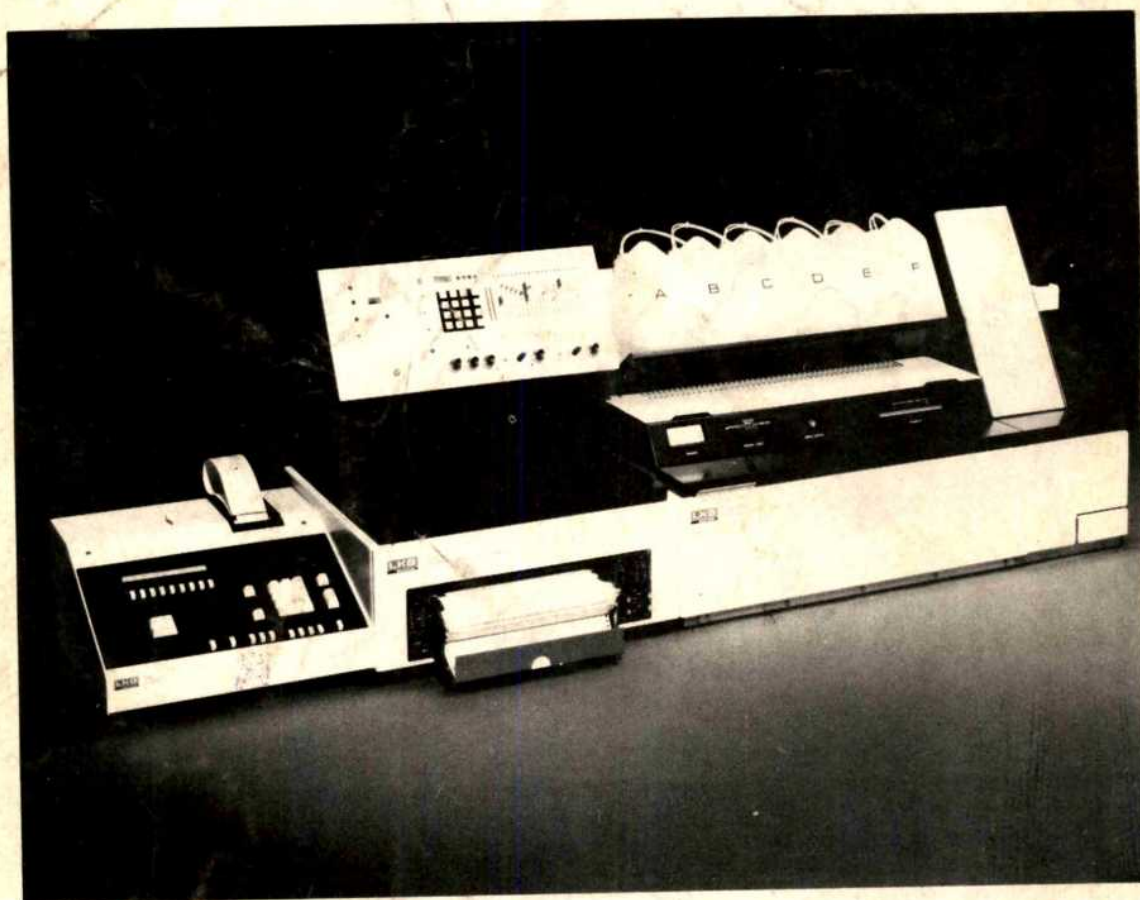
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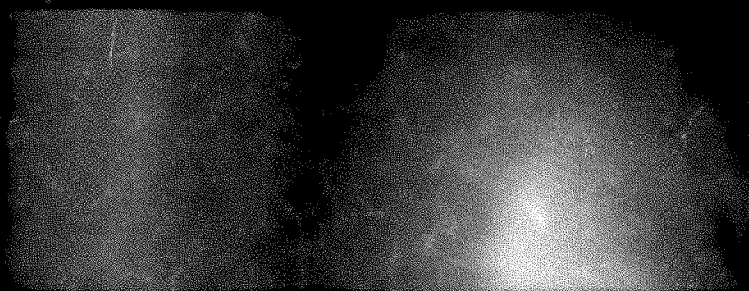


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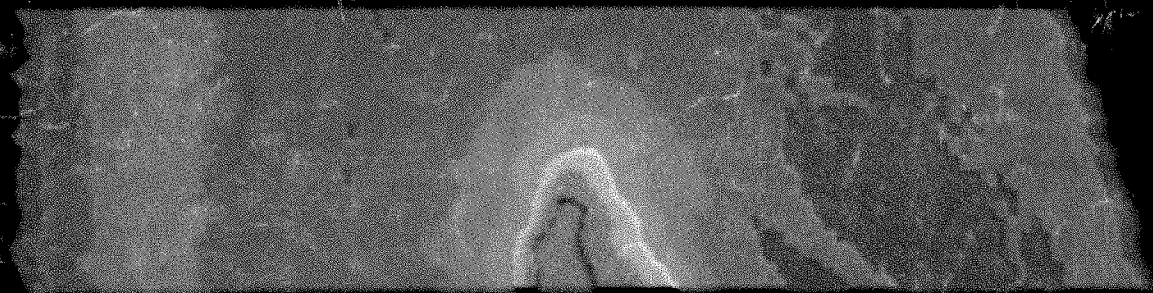
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ACHEMA Exhibition News

VENUS  
11.5 MICRON IR IMAGE



VENUS IR CLOUD MAP



■ 215 K  
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Pioneer images  
of Venus clouds



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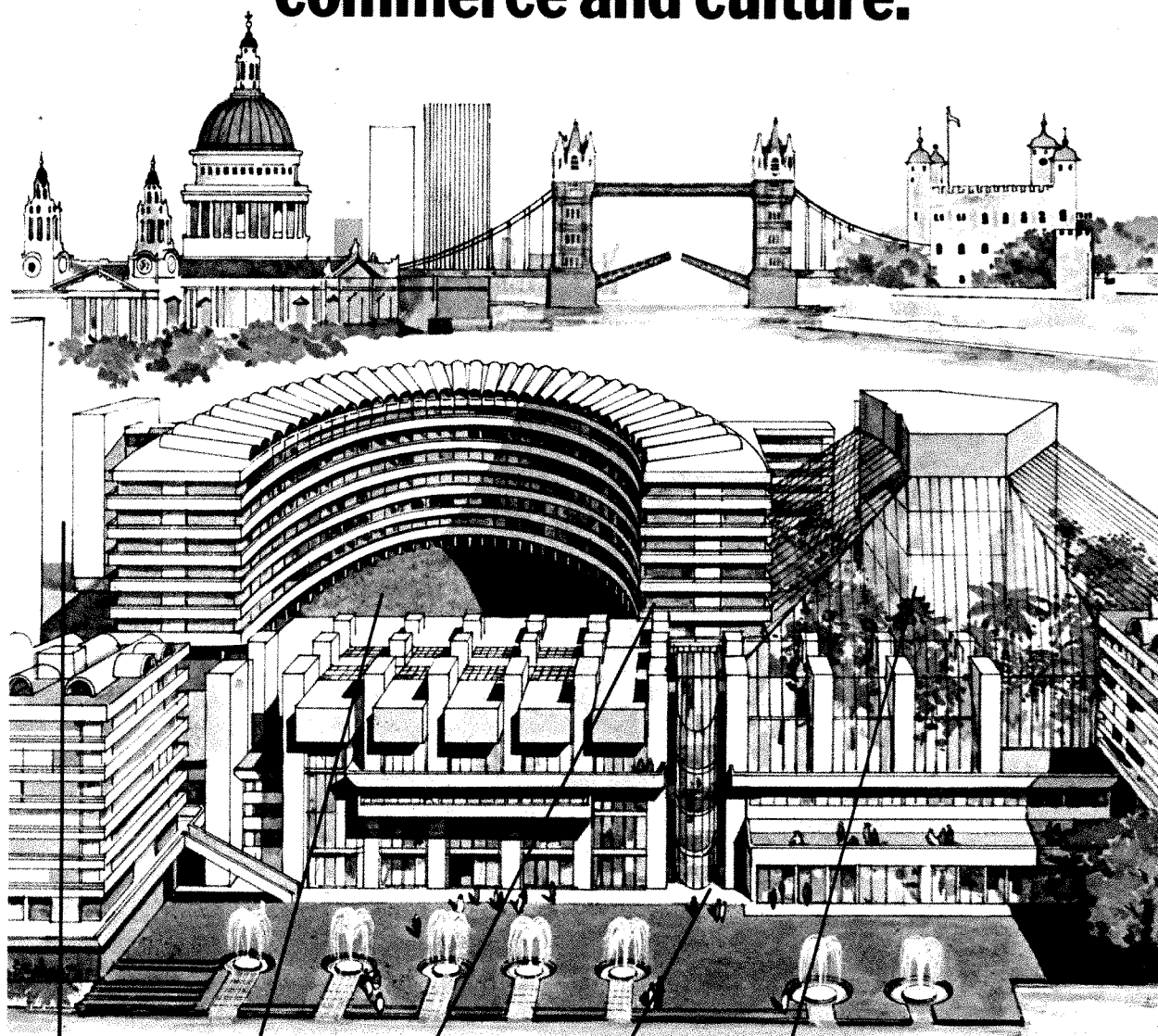
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Top, High-resolution 11.5- $\mu$ m image of thermal emission from cloud tops near Venus' north pole, from Pioneer Venus Orbiter on 15 Dec 1978. The bright (warm) region is a clearing in the clouds and the dark regions represent a high circumpolar cloud band. Bottom, Intensity-sliced, false-colour version of the image showing temperature values. See page 613. Other papers on Venus are on pages 614, 616 and 618.

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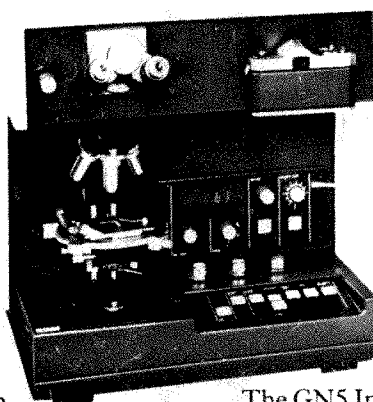
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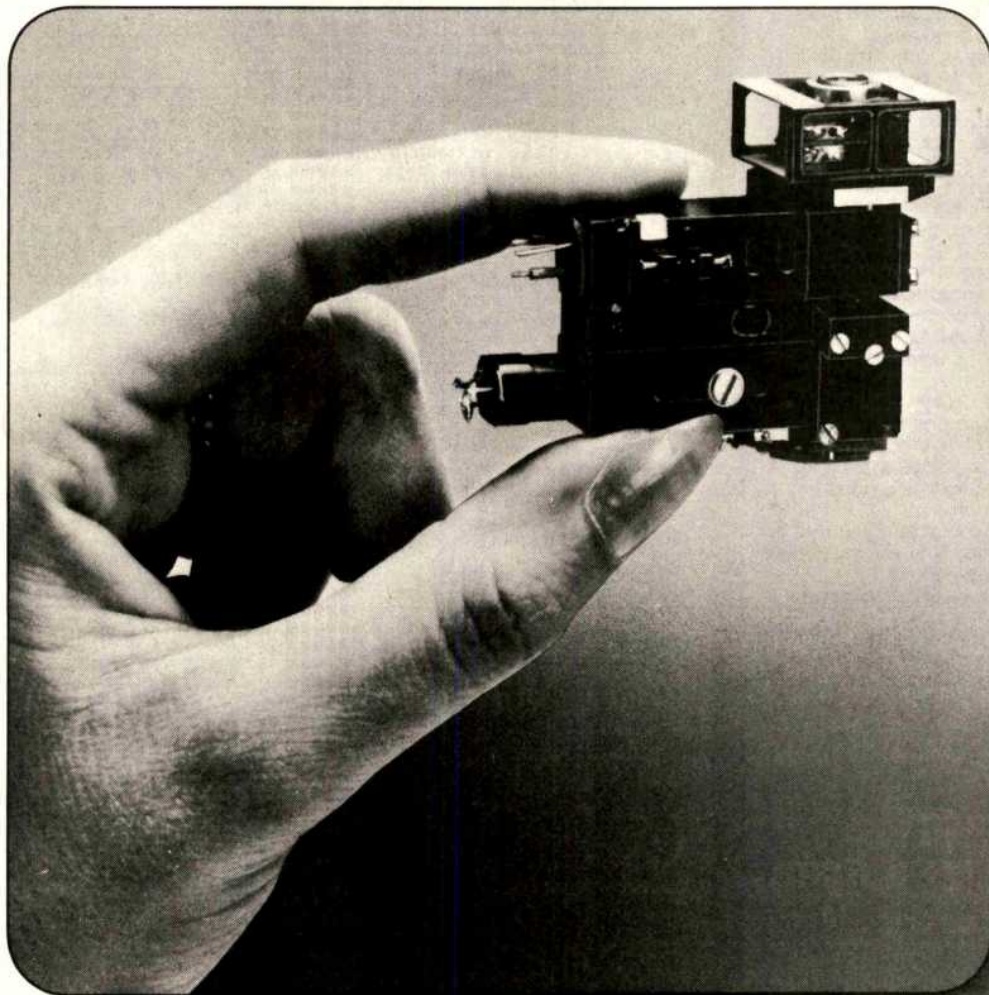


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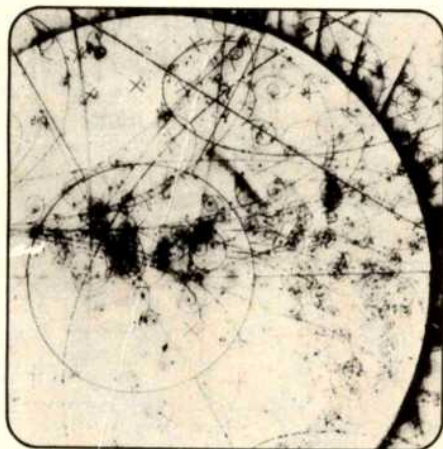


contained within the ultra-compact head.

Light output from the laser is split by the optical system into two beams: 90 percent intensity for recording and 10 percent for reading. The read beam passes two mirrors and a beam splitter that are arranged to recombine the record and read beams at the objective, which focuses onto the information layer in the disk with an accuracy of  $1\text{-}\mu\text{m}$ .

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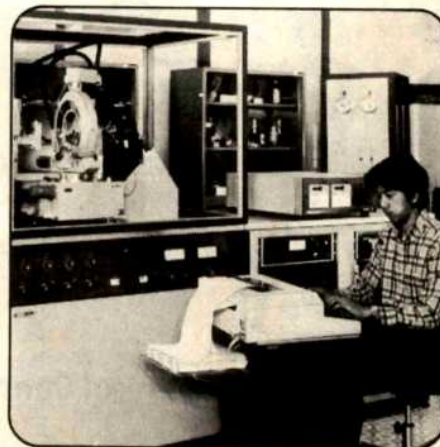
Diode laser optical recording is one example of Philips' ability to translate advanced technology into practical innovation. Here are some others.



CERN, the Geneva-based European Organisation for Nuclear Research, is to install four 500kW, 200MHz power amplifiers for their Super Proton Synchrotron, which is currently operating at a level of 400GeV. The new power amplifiers will provide additional RF power to double the Synchrotron's present capacity to facilitate experiments in high energy physics. Each of the four amplifiers employs 17 air-cooled coaxial power tetrode tubes of metal ceramic construction; power output per tube being approximately 37.5kW. The contract, awarded to Philips, calls for the supply and installation of the amplifiers as well as supply of spare tubes for a period of ten years.



**Fibre optics transmission.** Trials on a long haul fibre optics telephone transmission route, covering a distance of ninety-six kilometres, has been successfully completed at the Philips laboratories in the Netherlands. Essentially, the route comprises a 19mm diameter cable containing six 0.1mm fibres with a regeneration stage every eight kilometres.



**Tokyo Institute of Technology** has installed a Philips PW1100 computer-controlled Single-Crystal Diffractometer to assist in structure analysis of inorganic materials. One of Japan's leading universities, the Tokyo Institute of Technology, is noted for its academic research into the field of inorganic materials. And the new installation will be used by crystallographers from all over the country.

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**nature**

14 June 1979

## Not the percentage but the cash

How much should a nation spend on research and development? Advanced industrial countries generally devote between one and two per cent of their gross domestic product to R & D; usually somewhat more than half of the figure will come from government sources, the rest from private investment. Recent OECD figures (*OECD Observer* No. 97) show that Britain, devoting 2.1% of its GDP to R & D, still seems satisfyingly committed to science and technology. The reality is somewhat different.

Britain's GDP is (on a per capita basis) little more than half that of many other industrial nations. So if we ask how much money per capita is spent on R & D, Britain's position looks decidedly less distinguished. Whilst the US spends \$200 per capita annually, Germany \$176, Sweden \$171, the Netherlands \$146, France \$130 and Japan \$103, Britain only manages \$92. Comparisons look even less happy when an allowance is made for defence R & D in which the United Kingdom is a heavier spender than almost any other nation. The conclusion has to be that in civil

R & D expenditure the UK is quietly losing touch with the rest of the industrialised world; not through any visible lack of enthusiasm for science and technology but simply through lack of resources.

Talks of cuts in public expenditure are very much in the air at present as a new Conservative government tries to live up to its election pledge of tax reductions. On the other hand, the Advisory Board for the Research Councils recently made a strong plea for 4% real growth in science expenditure in the coming years. The many government departments with significant commitment to R & D will find it easiest to go for a middle path—no real growth. But in another ten years our relative scientific position will then be much worse and scientists will be taking every opportunity to emigrate.

There is no guarantee that a stronger commitment to science and technology will boost the economy—but there hardly seems any doubt that support which is relatively weaker on an international basis will do permanent harm to the nation. □

## More on your fuel bill

THE winter in Britain is now over. Any further freak snow-storms, freezes, high winds and flooding should be attributed to next winter; this past winter, by general consensus a miserable one, cannot be blamed indefinitely. There is one final reckoning, however: the last quarter's gas and electricity bills sitting unwanted on doormats around the country.

These bills, everyone must know, are produced by computer. How else would we have a line reading 'VAT at 0% on £72.18=£0.00'—or a 52 digit code number sternly marked 'for machine use only'? But what is surprising is not what the computer does to embellish these straightforward demands for money—it is what it leaves out.

Every organisation devoted to the selling of energy is at least nominally also interested in conserving the stuff. Conservation, however, is not just a question of double-glazing and thick sweaters; it is, or at least it should be, yet another part of that monster of the 1970s—the information industry.

The most valuable piece of information that any energy consumer can have at present is how conservation measures have cut down fuel bills. Did recently installed insulation in the attic have any perceptible effect on the

use of fuel? If so, that little bit of information, passed round from person to person, is a much greater incentive to others to invest in conservation measures than is any advertisement proclaiming the merits of insulation. If on the other hand fuel is not conserved thereby, then the consumer should equally be alerted that all is not well.

Presumably within the computers of the utilities there is information on each consumer's energy usage over a period of many years. This by itself would not be adequate to allow for weather fluctuations. But someone, somewhere must be collecting rudimentary data on how cold it is—such as the simple but valuable 'degree-days' measure widely publicised in the United States. It is surely not beyond the wit of the energy utilities to provide a line on each bill giving, say, the number of units consumed per degree-day for the quarter in question—and corresponding figures for the past five years.

No doubt there can be endless discussion about the merits of various units, the possibility that consumers will misinterpret the figures, the fraction of fuel that is used for heating and so on. But anything would be better than today's total lack of information. And there is room on the bill—that line about  $0 \times £72.18 = 0$  could go for a start. □



# Problems with the shuttle cause concern for US space science programmes

David Dickson reports from Washington

TECHNICAL problems and the related cost over-runs of the National Aeronautics and Space Administration's space shuttle programme are raising serious concerns about the possible impact on planned space science missions. So far, no major changes in these missions have been necessary. But with performance margins being squeezed increasingly tightly, contingency plans are already being drawn up for two planetary missions that depend on space shuttle launches—one to send a pair of space vehicles to Jupiter, and the other to send two vehicles to the Sun—in case any further difficulties arise.

Equally important in the medium term is the effect the current shuttle problems could have on new space science programmes. At present Congress seems prepared to accept that the military need for the shuttle is sufficient to justify paying the increased costs recently announced by NASA; but whether it is also prepared to support new space programmes remains uncertain.

Senator Adlai Stevenson Jr., chairman of the Senate subcommittee on science and space, warned at a hearing of the subcommittee last week that the shuttle problems could require an extra \$500 million a year from the US taxpayer to maintain a civil space programme. "And that may prove to be too high a price to pay," he said.

The most direct manifestation of the shuttle's problems have been the delays caused by problems in engine testing and, more recently, in fixing the heat-protective tiles to the outer skin of the orbiter. NASA officials now admit that the shuttle is unlikely to be airborne before next spring, perhaps even as late as the summer: and Dr Robert Frosch, NASA administrator, told the Senate subcommittee that total cost overruns for the design, development, test and evaluation part of the programme were now expected to be about 15%—or roughly \$1 billion in current dollar values—compared to a 7% estimate last year.

The engine problems, however, have also had effects on the programme. For example the reduced performance estimates for early shuttle launches have led to erosion of engineering and weight margins in space science payload design. One programme to have been affected by this is Project Galileo, a twin probe and orbiter due to be launched from the shuttle in 1982, the former to provide the first direct samplings of the planet's atmosphere,

the latter to transmit data from an elliptical orbit through all regions around the planet, including at least 11 near encounters with its surface.

The complexities of the manoeuvring required of the two vehicles on reaching the planet have resulted in a slightly heavier design than first anticipated. Initial fears that this might require discarding some of the scientific experiments have now been circumvented; but the extra weight together with the reduced performance expected of the early space shuttle flights, have required significant design changes in the so-called Interim Upper Stage (IUS) which will launch the Galileo vehicles from the shuttle.

At present, assuming that the IUS development goes as planned, performance margins appear to be acceptable. "We seem to have got the weight problem under control, and the project itself looks in pretty good shape at the moment," project scientist Dr Torrence V. Johnson of the Jet Propulsion Laboratory in California said last week.

Any further problems, however, either in the IUS development or in the shuttle test schedule would be a serious matter. At present Galileo, which will be among the first operational shuttle launches, is planned for early 1982, to reach Jupiter in the middle of 1985. On this schedule the spacecraft would fly close to Mars,

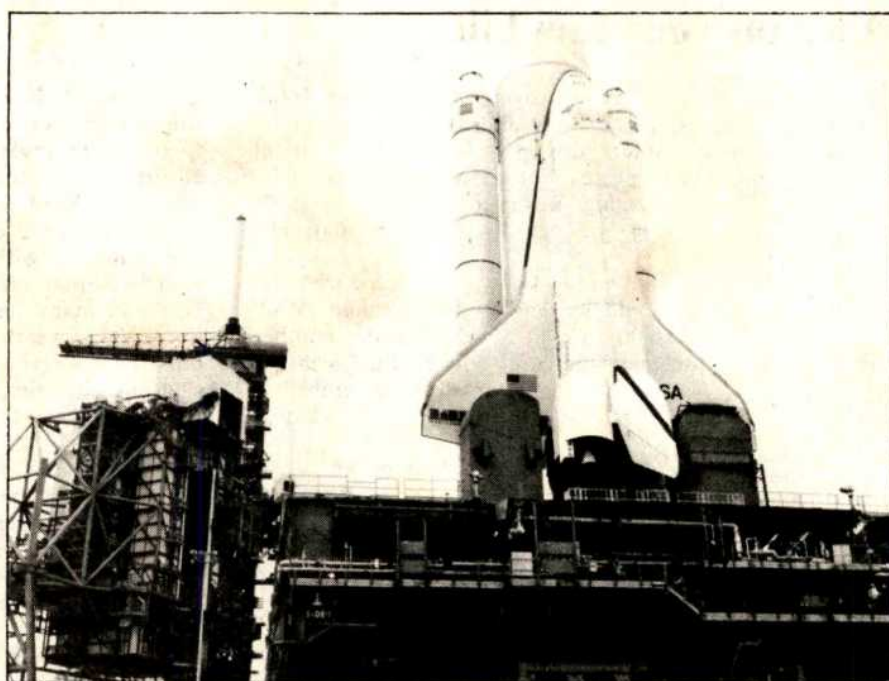
using that planet's gravitational pull to help it on its way.

A major delay in the shuttle programme, however, pushing the Galileo launch into 1983, would mean that the gravitational pull of Mars could not be used. Extra fuel would then be required to get the spacecraft to Jupiter, and some of the scientific projects would be dropped to compensate for the extra weight.

Various contingency plans are already being studied by NASA. One possibility might be to split the mission into two halves, sending the orbiter and the probe separately, perhaps a year apart; but this would involve considerable extra cost, and a major redesign.

Another project similarly affected by weight considerations is the international solar polar mission (ISPM). This is a joint project between NASA and the European Space Agency, due for launch at the beginning of 1983, which will involve sending two spacecraft simultaneously past Jupiter and into opposing orbits over the poles of the sun. Some of the potential weight problems for this mission have been compensated for by changing the trajectory of the launch from the shuttle. But the margins are now very tight (technically the ISPM is now 70 pounds—or 5% overweight, compared to 146 pounds for Galileo); and any problems with the IUS could have severe consequences.

As a contingency plan, NASA has



Space shuttle: setbacks are keeping other projects firmly on the ground



suggested that the two vehicles might be launched separately, possibly up to a year apart. But ESA scientists are not very happy with this suggestion, pointing out that a simultaneous launch is one of the attractions of the mission.

Weight problems are also causing concern to planners for the first Spacelab flight. This is still scheduled to take place in August 1981, a date that ESA officials are confident can be met even with considerable slippage in the shuttle test programme.

However as things stand at present, the shuttle may not be ready to provide enough thrust by this stage to launch the full Spacelab payload. The current shortage is about 1,300 pounds out of a total weight of about 65,000 pounds; and it could mean that some of the experiments already lined up for the first Spacelab flight may have to be dropped. (One encouraging piece of news to ESA officials is that the US Air Force appears to be planning to use Spacelab for some of its own manned space-flight missions.)

The space shuttle problems are already affecting other areas of NASA's activity. Scientists involved in the Voyager mission, for example, feel that if it had not been for these problems, they might have had access to some extra money this summer for additional experiments.

The greatest uncertainty at present, however, is over what the political effects of the shuttle's problems will be on support for future projects. NASA, with the support of both the Office of Science and Technology Policy and the Office of Management and Budget, is adamant that none of the existing programmes should be cut to provide the additional funds needed by the shuttle.

Dr Frosch told the Senate subcommittee that cancelling the Galileo project—at one time discussed as a distinct possibility—would only save \$83 million in 1980, considerably less than the extra money needed by the shuttle; nor would much be gained by taking money from the space telescope. "We do not consider either of these reasonable ways of getting extra funds," he said.

But the concern is that Congress, having provided NASA with the extra shuttle costs largely on the grounds that the success of the programme is required for US Defense plans—in particular for launching surveillance satellites, will be reluctant to complement this with support for new space science missions.

The situation is already bleak following the decision of the Carter administration not to request any new starts for the fiscal year 1980 partly to ensure adequate support for space

shuttle. (Among projects which NASA had submitted to OMB were plans for a shuttle-launched gamma-ray observatory, an orbiting imaging radar to Venus, and a mission to the comets Halley and Enke.)

"The real question is going to be what the 1981 budget contains. A second year with no new starts could really put the science programmes in jeopardy; if the nation wants a vigorous space science programme, then you have to start something new," Professor A. Cameron of Harvard College Observatory, chairman of the National Academy of Science's space science board, said last week.

Of course, if there are no new starts in the 1981 budget request the fault will not lie entirely with space shuttle; equally important may well turn out to be President Carter's desire to greet an election year with an attempt at a balanced budget.

But the additional funding which NASA is currently seeking from Congress for 1980 (\$225 million above its original request), has not improved the situation. "We are still hopeful that our project will be funded, but we are not as optimistic as we were a month ago," says one of the scientists involved with the gamma-ray telescope, currently top of the NASA's space astronomy and astrophysics board's priority list for future projects.

The dangers in the current situation have not been lost to Senator Stevenson who, with fellow committee member and ex-astronaut Harrison (Jack) Schmitt, has been urging a more aggressive space programme on the administration for some time.

"The problem with the shuttle programme now seems to have been a serious underfunding in the early stages, something of which up to six weeks ago the Congress had been unaware. And now it has all come home to roost with a 15% cost over-run," Mr Stevenson said at the subcommittee hearing last week.

"The biggest cost may effectively be the end of the civilian space programme, since the real risk is that the price of the shuttle's difficulties will be taken out of the civilian side of our space efforts. And with the country in its present mood, this could well happen."

Not all observers are quite so pessimistic. But the problems raised for future space science funding are already being taken seriously, not only by NASA but also by both OSTP and OMB. And they will provide an unwelcome house-warming present to greet NASA's new associate administrator for space science, Dr Thomas Mutch, when he takes up his appointment next month. □

## California plans to build world's largest telescope

THE University of California is in the midst of preparing preliminary designs for a 10-metre diameter optical telescope that planners hope will be operating in the mid-1980s. Two designs are being considered, each a radical departure from conventional optical telescopes, which would be prohibitively expensive to scale up to this size.

The proposed telescope would be by far the largest in the world, with a mirror twice the diameter of the Hale telescope on Mount Palomar. (Nominally the largest telescope in the world is now the 6m at Zelenchukskaya Astrophysical Observatory in the Soviet Union, but western astronomers generally comment that it has not yet been proved reliable.)

Jerry Nelson, of Lawrence Berkeley Laboratory and chairman of the technical design committee for the proposed telescope told a meeting of Californian science writers last month that it would be designed to produce images smaller than 0.5 arc seconds and that, for good infrared observations, it would have to be built at a site with low atmospheric moisture.

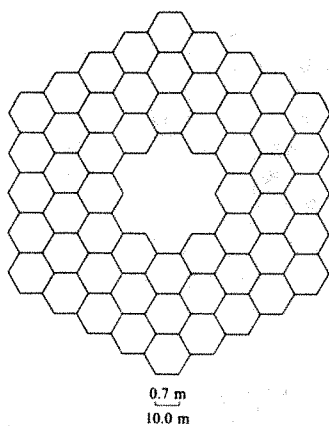
Historically, the costs of optical telescopes with thick, monolithic glass mirrors have risen roughly as the cube of the diameter. This means that a conventionally designed 10-metre instrument would cost around \$200 million—a figure Nelson says is "out of the question". On the other hand, the design committee has already rejected such alternatives as the use of independently focused multiple mirrors, like the recently opened telescope at Mount Hopkins (*Nature*, 3 May, page 9). Such multiple mirror devices may provide an economical alternative for studying point sources, but the new University of California telescope must have angular field wide enough to encompass an extended image like a galaxy with associated star clusters.

Nelson's design committee has thus narrowed the acceptable options down to two: a single mirror much thinner than those used previously, or a segmented mirror with focusing characteristics approximating those of the monolithic design. In either case the anticipated principal focal length would be between 15 and 20 metres, which would facilitate a relatively small containment structure. Traditionally half the expense of building a new observatory has gone into the telescope and half into the surrounding structure, so every effort is being taken to minimise the overall size.

The instrument will also be made more compact by the use of an altazimuth mount. Most large telescopes have been mounted with their axis of rotation parallel to that of the Earth, so that only one sweeping movement is required to track an object across the sky, but this so-called equatorial mount is relatively bulky. The advent of computer control has now removed the obstacles that once prevented use of the smaller but more complex altazimuth mount, which requires coordinated rotation about both the vertical and horizontal axes.

For either the thin continuous mirror or the segmented mirror, the question of how to evenly distribute the weight of the glass raises a host of unsolved problems. In either case 'active optics' will be required—that is, the stress on various support points must change continuously to prevent deformation of the glass when the mirror is tilted. The thin monolithic mirror would require some 100 to 200 support points, the majority of which must be active. On the other design, each segment of the composite mirror would require 12 sensors and 3 actuators to change its tilt and focus. On this point, at least, Nelson seems confident. "We think control is no problem," he says. A simple actuator device for segments of the composite mirror has already been tested and shown to be many times more precise than required, and the overall control scheme has been successfully modelled on a computer.

Preparing the glass, however, is another matter. A leading glass company has agreed to prepare the monolithic blank if that design is finally chosen, but many problems would remain in caring for the world's largest piece of fragile glassware. Among other things, the whole mirror, planned to be only 15 centimetres thick,



Possible segmented design for 10m mirror

would have to be enclosed in a vacuum tank each year for re-aluminizing. The segmented mirror also under consideration would require nine basic segment shapes to produce an overall parabolic curvature. The polishing might be done by computer control on segments already prepared for mounting, or possibly by a cheaper method—which Nelson calls "extremely promising"—in which the pre-stressed segments are polished spherically then released to snap back into the shape of an off-axis parabola. The ten centimetre thick segments would be hexagonal, 0.7 metres on a side, and mounted in three rings around a larger central mirror. The completed composite mirror would contain 54 segments.

The final cost should be in the range of \$20-50 million, of which the university hopes to raise at least half initially and then try to obtain matching federal funds. If construction can begin by 1981 the new telescope might be completed by 1986.

To avoid as much water vapour as possible, three relatively arid mountains have been proposed as possible sites. Mauna Kea, a 14,000 foot volcano in

Hawaii, would be the most accessible since the roads already lead to existing observatories on its peak. White Mountain in California, also 14,000 feet high, would be closer to the university's campuses, but the summit is almost unreachable by land vehicle. Junipero Serra Mountain is close to the UC Santa Cruz campus, which has a particularly active astronomy department, but with a height of only 6,000 feet it is by far the most moist of the three locations. Wherever the new observatory is built, the planners have already resigned themselves to building a structure sturdy enough to allow observations in the midst of 50 mph winds.

Nelson and others involved in the project sound optimistic about being able to build their proposed observatory, despite its high cost and necessarily radical design. They point out four broad classes of observation that would be facilitated by the proposed instrument:

- With direct optical imaging, a 10-metre telescope would permit better study of distant galaxies and a further attack on the problem of whether the universe will expand for ever or eventually collapse.
- Direct infrared observations would be used to study protostars and the structure of the galactic centre—perhaps resolving the question of whether it contains a massive black hole.
- Optical spectroscopy using the new instrument would allow astronomers to study globular clusters around other galaxies and investigate why quasars have absorption lines.
- Finally, infrared spectroscopy could be used to determine the abundances of various molecules in stars, and to study the emission spectra of quasars.

John Douglas

## Sussex students step up science exam protest

PROTEST escalated last week at the campus of Sussex University against a compulsory first-year preliminary science examination. Two students, Richard Flint, president of the student union and Shaun Fensom, an engineering student, were "excluded permanently" from campus by vice-Chancellor Sir Denys Wilkinson for their "leadership" of a student-union-mandated disruption of a resitting of the contested examination.

An emergency Senate meeting has been called for 13 June when the Vice-Chancellor will explain his action to the faculty.

Half the university's 4,000 students attended an emergency meeting on 5 June. They rejected a call for an

immediate occupation of university buildings, but voted to canvass university and staff to protest against the expulsion and seek support from campus trade unions. They also decided on a series of one-day strikes the first of which was held last Friday with mass picketing at university entrances. The vice-chancellor was turned away. Students estimated that attendance was reduced to 10%-20% of normal but administration officials claimed that the action had not achieved its aim of stopping all classes.

The protest is taking a form not familiar since the 1960s. The original campaign began two years ago when the student union voted for direct student action to end a compulsory

first-year examination in science subjects. The science division is the only one at Sussex with such a requirement. Arguing that the exam distorted teaching practices and served no useful assessment purpose, the union called for a boycott. In the first year the boycott was 65% effective.

In the meantime a nine-member faculty working party was set up to consider the students' demand. It rejected the students' proposal that the exam be made optional, citing a need for "rigorous training in discipline", "an ability to marshal facts under pressure and to meet deadlines" and the "necessity for an objective means to monitor progress" as reasons for its decision. The working party specifically

rejected the students' request for tutorial assessment.

Students maintained their position, arguing that skill at examinations is unrelated to the skills it takes to be a competent scientist and that gearing the course towards quantitative assessment actually reduced learning. In January, a second boycott was called, with the addition that any

resitting of the exam that included boycotters would be disrupted.

The second boycott was far less successful with only 10% of the students failing to attend. Nevertheless the university rescheduled the exam to include students who had participated in the boycott. The exam was disrupted by 25 students who occupied the room. A second resitting was re-

scheduled, this time with faculty, police and photographers in attendance, but the students banged on tin cans and drums outside the examination room.

On 1 June, the two students were summoned to the vice-chancellor's office and expelled for their part in the disturbances.

Joe Schwartz

## Soviets get their fun from learning about science

SOVIET society is in many ways surprisingly Victorian—a trend shown in the great popularity of the scientific lecture as a form of cultural entertainment. The organisation of such lectures is the task of the *Znanie* (Knowledge) Society, founded in 1947, which, in Soviet society, plays roughly the role of the British Association for the Advancement of Science, the Royal Institution, and the Worker's Educational Association in the United Kingdom.

Recently, two notable representatives of *Znanie*, Nobel Laureate Academician Basov and Dr Vladislavlev, toured the UK as part of the "Days of Soviet Science and Technology" organised in connection with the Soviet Exhibition at Earls Court. A delegation of 11 scientists and some 10 support personnel took part in the "Days". The host was the British Association—although, by some failure in communication, they were not aware until the delegation arrived of the great significance which Soviet science publicity puts on the organisation of such "Days". Nevertheless, one outcome of the visit was agreement to exchange lecturers between *Znanie* and the British Association—although it seems unlikely that the Soviet public will have the direct benefit of words of wisdom from the BA in the foreseeable future.

For *Znanie*, although lauded in official handbooks as being built on "democratic principles", is a hierarchical organisation. Although the society is supposed to organise lectures for any Soviet collective farm, factory or club that demands them, it would be impractical for top-flight lecturers to be constantly commuting, say, between Moscow and Vladivostok. "What we do", explained Vladislavlev "is to organise 'lectures for lecturers' in such remote places. Then the lecturers we have trained can give lectures at the local level".

If a top specialist is required, then the organisation requesting him must pay his fare. This suggests that the remote areas are somewhat dis-

advantaged—an idea which the visitors resolutely denied. As far as the Virgin Lands, and the far north of Siberia are concerned, they explained, there are special funds—and for the rest, there are no problems. Local lecturers are well trained to do their job in popularising science.

Lecturers, in fact, form the only 'members' of *Znanie* (apart from administration personnel). The main privilege of membership is to give frequent lectures (usually gratis) to whatever audience demands your services, and to attend frequent seminars on the latest developments in your field and the newest methods of lecturing. "The main problem", Vladislavlev explained, "is to make lectures appropriate for different audiences. That is why it is obligatory, when you ask for a lecturer, to describe the intended audience."

Not all lectures are simply a cultural diversion. *Znanie* is also responsible for the 'People's Universities', a fairly new feature of Soviet life. Courses are organised by plants and ministries to provide workers with the means of improving their professional qualifications.

*Znanie* itself is only concerned with the academic side—it is up to the factory commissioning its services to provide the facilities and to organise funding. Some 60% of *Znanie* funds, he added, come from non-state sources—factories, and trade unions. Of course, if a worker simply want to study, say, English Literature, he added, "then he pays for himself, just as if he was going to the cinema!"

To the Soviet mind, 'science' implies, as Academician Basov put it, "all advances in knowledge". Thus the 'flow-diagram' of *Znanie's* work includes 'Houses of atheism' in parallel with its planetaria. The most popular lectures organised by the society, according to Vladislavlev, are those on international affairs, followed by medicine.

Only the lecturers and organisers, not the audiences, are members of *Znanie*. If, say, some Ukrainian collec-

tive-farm wants to start a 'Bird-watchers' club', *Znanie* will provide lecturers and suggest reading matter, but the club cannot become an associate member of *Znanie*. Only high-level professional societies, such as the "Popov Radio-technical Society" has that privilege. (Some 40 such societies at present are 'collective members' of *Znanie*.)

Even so, with more than three million active members (lecturers and paid local organisers), there is no need to invite further people to join. "We have too many members already", Basov said. Some prominent personalities, of course, are virtually *ex officio* members. All Soviet cosmonauts, said Vladislavlev, are exploited enormously. "In fact, their bosses are always telling us to leave them in peace to get on with their job."

"There is an increasing demand for scientific knowledge and an increasing interest" said Vladislavlev. "The more one knows, the more one wants to know." As for the social impact of science, Academician Basov reiterated an earlier communication to the British Association that "science is not always good!" It would appear, however, that, in spite of a vast quantity of general science reporting (all major discoveries are reported in *Pravda*, Basov said), the Soviet public have not developed a "doomwatch outlook".

"It is *not* disasters that interest the public in science", said Vladislavlev, referring to Harrisburg. "The interest comes naturally from the rising cultural level."

And, as far as the Soviet citizen is concerned, everything is done to encourage that rise. "First we offer him a single lecture", said Vladislavlev. "Then, having caught his interest, a course of lectures. And finally, enrolment in a People's University. We are concerned in what you would call continuous adult education, and its development is our main task for the future!"

Vera Rich



## news in brief

**MRC staff committee prepares for budget fight:** Responding to a leak in *The Observer* (3 June) predicting extensive cuts in UK science in this week's budget, the Medical Research Council Staff Side Committee initiated actions last week to try to prevent the cuts. Petitions and telegrams from every MRC unit have been sent to Neil Macfarlane, Minister of Science, protesting cutbacks in research funding. Eight of the nine trade unions represented on the staff committee supported a move for an immediate meeting with Macfarlane but the minister refused the request calling it "premature". Staff side initiatives to make joint representations to the government with MRC management were similarly rebuffed last week. In a letter, MRC management rejected the possibility of joint action opposing budget cuts saying that management and staff should act independently since the two have different views. The full staff side committee will meet 20 June to make its long term plans to prevent damage to medical research in the UK.

**US scientists report successful laser fusion tests:** Scientists at the University of Rochester in New York suggested last week that the highly successful results of a series of experiments on a laser fusion energy system might bring forward the date at which break-even in fusion energy—the point at which the energy produced by the system equals the energy inserted—can be achieved.

The experiments were carried out using a six-beam ZETA laser system, the first stage of a 24-beam system scheduled to begin operation later in the year. Using a laser power level of  $1.65 \times 10^{12}$  watts, the experiment generated more than one thousand million neutrons, at temperatures of 67 million degrees. "This is between five and ten times the amount that was expected," Dr Moshe Lubin, director of the university's Laser Energetics Laboratory, said last week.

When the full 24-beam system is in operation, it is hoped to be able to produce 30 to 40 terawatts ( $\text{watts} \times 10^{12}$ ) of power. "The success of our first experiments means that it may be possible for us here at the Laser Energetics Laboratory to help the national programme move up the date that is projected for breakeven. That event is presently estimated to occur in the 1984 timeframe," said Dr Lubin.

**Third World energy experts to confront the West:** A symposium to be held in London 20–22 June will provide a forum for 30 spokespersons from the Third World to confront "the dominance being exercised by developed countries in science and technology, monetary expenditure, and planning and policy." The conference, titled Third World Energy Strategies and the Role of the Industrialised Countries is being organised by Ariane van Buren and Gerald Foley of the International Institute for Energy Development and is designed to give experts from the Third World a chance to address influential figures from western aid agencies and development banks in the areas of nuclear power, the role of multinational oil companies in development and the desirability of the alternate technologies currently in western vogue as being "appropriate" for the Third World. Papers will be pre-circulated and speakers will be restricted to five minute presentations thus allowing for up to one hour of debate. The papers and debates will be published. Sponsors of the meeting include the UK Ministry of Overseas Development, Atlantic Richfield, and the Beiger Institute of the Swedish Academy of Sciences.

**UNCTAD bodes ill for UNCSTD:** The increasing economic crisis facing the developed capitalist countries has produced a complete deadlock at the fifth UN Conference on Trade and Development. Four weeks of negotiations at Manila among 5,000 delegates from the world's rich and poor nations costing an estimated £100m have failed to reach agreement on any restructuring of the western world's economy. "The resolutions passed commit us to absolutely nothing" said one western delegate. Third World countries attempted to change current practices of the International Monetary Fund, to install a system of trade preferences and multilateral trade negotiations for developing countries and to break the industrialised nation's monopoly on shipping. Final resolutions condemned protectionism in trade against Third World products, provided for short and long term "action programmes" and expressed a need to progress towards an aid target of 0.7% of GNP. But several countries, including the UK, said they could make no commitment due to current cutbacks in public spending. The failure of the rich nations to yield to Third World demands for economic change reduces the possibility for effective action to be taken at the UN Conference on Science, Technology and Development to be held in Vienna in August.

**India extends ban on wildlife export:** The Union Ministry of Commerce has restricted wildlife exports to only 21 live species of birds, one live species of animal and products of six other animals, on the recommendation of a sub-committee of the Indian Board for Wildlife which examined the existing export policy from an ecological viewpoint. The only animal species that can now be exported is the striped squirrel; allowed bird species include myna (other than hill myna), parakeet, and two Himalayan species known for their colourful plumage—Sibia and Siva. Among the allowed animal products are the skins of common fox, hill fox, jackal and jungle cats, and peacock feathers and porcupine quills. The new policy follows an import-export order of January 1978 which banned most of the wildlife in demand abroad, and the recent revision will further hit the trade. The bird trade is worth 80 million rupees annually, the major portion being export: according to a recent report of the Royal Society for Protection of Birds, 4.5 million birds used to be exported from India every year. Export of munias, constituting 70% of the total bird trade, was banned last year, and the ban on the export of rhesus monkey (about 20,000 annually) and on the lucrative trade of pelts and furs of cat family and skins of lizards have drastically affected the animal trade. But there has been a concurrent increase in poaching and smuggling of wildlife and their products. Recently a global racket involving the

exchange of wrist watches and other luxury items for snake skins was uncovered in Bombay. So although conservationists and naturalists are pleased by the recent directive—except for jackal skin, an animal fast decreasing in number—they feel much more rigorous policing is required.

from Dilip M. Salwi,  
New Delhi.



"Keep your part of the bargain.  
Throw out the wrist watches!"



# Commercial break for Britain's science?

THE word 'atmosphere' liberally peppers the speech of Neil Macfarlane. It is used constantly in attempts to describe just how important good morale is for the country's scientific community, and in particular, the newly-appointed Minister for Science believes there must be infinitely better links between "the research atmosphere" of the good universities and industry in Britain.

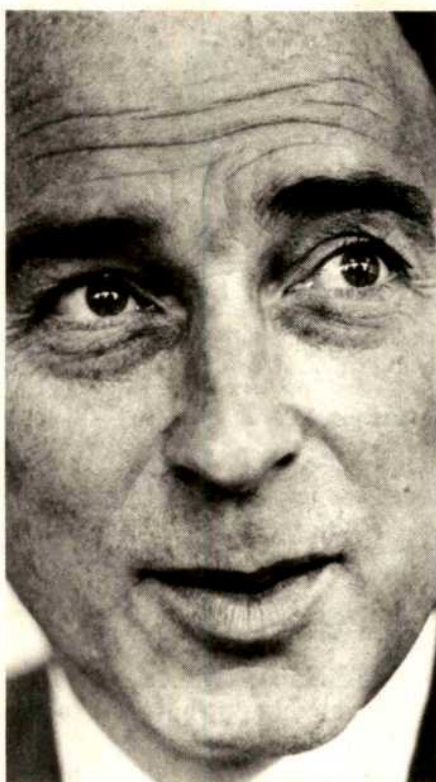
"We have lost a lot of ground over the past 20 years now", he states. "Too many industrial organisations have turned to their companies' research activities when looking for savings on their annual budget." To rectify matters, he maintains that we must encourage companies to set up centres close to universities—as the Americans do. Similarly our universities must be more commercially minded in looking for closer ties with major firms.

But 43-year-old Mr Macfarlane stresses that the real effort must come from industry, which in the past has shown little regard for scientific and engineering talent. "I think it is greatly to be regretted that the engineer and the scientist are generally relegated to a secondary level in the hierarchy of any major industrial organisation in the UK. We have been taken over by financiers and lawyers."

These are candid words from a junior minister who has no scientific training and whose background is solidly commercial. Educated at Bancroft's School, Woodford Green, he was commissioned in the Essex Regiment from 1955 to 1957. He left "after Suez, the Middle East and all that particular cauffle" for the oil industry, to eventually become a sales manager with BP; he was closely involved in the 1973 oil crisis, dealing with the distribution of petrol in Southern England.

In February 1974, he was elected Conservative MP for Sutton and Cheam and straightaway sat on the Select Committee for Science and Technology. His experience there opened up a completely new interest in science and Mr Macfarlane is quick to defend the committee's record for giving a good overview of science and technology and for its many short, sharp investigations: "I think the whole atmosphere which then developed because of the decline of our industry prompted many of us to pursue various technological issues with vigour and at all levels."

He is certainly no supporter of the proposal by the Select Committee on Procedure for the dissolution of the science and technology group. "I



Neil Macfarlane (above), Minister for Science in the new Tory government, wants closer ties between industry and research

Profile by Robin McKie

would be very apprehensive if it was entirely disbanded because I would envisage a tremendous amount of duplication of its work by other government departments." But defenders of the committee cannot look to him as an ally in their fight for its retention, because he maintains that, as a member of the government, he has no right to intervene in a purely House of Commons matter.

Even if the committee is disbanded, Mr Macfarlane will remain optimistic, believing that in general both parliament and government serve science well. "I just wish that we could have a little more motivation of other Members of Parliament towards the importance of science. There is not nearly the interest that there should be." He believes the previous Labour administration did not help by giving all responsibility for science to the Secretary of State for Education and Science, Mrs Shirley Williams, without delegating to a junior minister. Education became such a political football that it took up most of her time, at the expense of science.

"However, I am not going to

criticise her all the way because I think in many respects she did show a tremendous interest and grasp, and doubtless she would have liked to have spent more time on science. But now there is someone who can spare that time and has access straight through to the Secretary of State who is totally involved himself. We have got to ensure that the science fraternity knows it is not neglected by this administration."

This desire is reflected in the breadth of Mr Macfarlane's role, which covers science, research, technology, engineering and preparing for the advent of the microprocessor revolution. Although there have been junior ministers for science before, they have not been faced with the same sweep of responsibilities.

One major problem is staff stagnation at university science departments, which is freezing promotions, preventing staff exchanges between centres and hindering the cross-fertilisation of ideas. Mr Macfarlane refuses to be pessimistic, however. "I don't entirely take a gloomy view of staff stagnation. It was probably bad eight or 10 years ago, maybe even four or five but I believe there has been a great awakening over this problem in recent years. Things are beginning to move and there is this exchange and cross-fertilisation taking place. We have just got to accelerate it somehow."

In general, he foresees "a period of stabilisation" for universities, with the emphasis being placed on training the 16-19 age group to provide skilled technician manpower for our industrial re-birth. The research councils can expect to continue with the level of funding which has occurred in the past couple of years.

On the thorny question of lay and union representation on the laboratory safety committees the Dangerous Pathogens Advisory Group and the Genetic Manipulation Advisory Group, Mr Macfarlane takes the same steadfast line as his Secretary of State, Mr Mark Carlisle, in opposing such moves. "These two bodies are so highly specialised that I don't believe there is a role for anybody else on them at this embryonic stage of their activities."

The real problem is using science properly in our industrial growth. "The main thing at the moment is that the atmosphere is right in the science departments—whereas five to 10 years ago it was very different. What I want to do is make Britain proud of its science, and that is going to be quite easy because our science is second to none. It is the very best." □



# How French 'postdocs' are left out in the cold

UNIVERSITIES have stopped growing in France, as in other western countries, and young scientists who want to make careers in research are feeling the pinch as badly as anywhere else. One paradox of this situation is that, at a time when postdoctoral fellowships are becoming scarce, researchers are increasingly reluctant to accept temporary posts, and less willing to change jobs merely to continue in research. While the problem is international, its French version has some special idiosyncracies.

Research in France is not as university-based as in some other western countries. A major role is played by the Centre National de la Recherche Scientifique (CNRS), which despite its name is not a place but an institution, financing a large number of research laboratories throughout France. Some of these are located on university campuses and some in the Grandes Ecoles, while others are free-standing. A fairly close analogy would be with the MRC research units in Britain.

Purely academic research is financed partly through the Ministry of Education and partly through the Delegation Générale à la Recherche Scientifique et Technologique (DGRST), a body which fulfils some of the functions of the UK Science Research Council. The DGRST provides a limited amount of finance for hardware, a fairly generous supply of research studentships, but only a very sparse ration of postdoctoral fellowships, a function which largely rests with CNRS. But CNRS does not provide fellowships for academic departments, only for its own laboratories and those 'associated' with it (which together accounted for some 7,600 research workers and 13,400 technicians in 1977). Since it is becoming steadily more difficult for university professors to raise funds for postdoctoral workers, as described later, there is a risk of prolonged contraction of academic research as compared with the CNRS-supported laboratories. DGRST is not now, it seems, in a position extensively to support either university research or that in the Grandes Ecoles, in the way SRC does in Britain by means of research grants (which often include funds for graduate research assistants), research fellowships, advanced fellowships and the like.

In France, all academics with teaching posts, and also employees of bodies like CNRS, are *ipso facto* civil servants (*fonctionnaires*) and as such have permanent tenure. They do not need therefore, and indeed are not entitled, to take part in the state's unemployment insurance scheme, ASSEDIC.

ASSEDIC was designed to cater for workers in private industry or commerce; it has been the subject of much negotiation between government and unions, and its terms were most recently improved last March. It is now a thoroughly comprehensive scheme—with one yawning omission: it does not cover temporary research workers ('postdocs') in universities, Ecoles, CNRS laboratories or similar establishments. French law is quite firm about this: postdocs are neither *fonctionnaires* nor are they employees in private industry, and so they slip neatly between the available stools.

French postdocs were not particularly concerned about this state of affairs until 1971 or 1972, when scientific unemployment first became noticeable. Pressure then began to be exerted on behalf of the postdocs by their

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**An unfortunate quirk of French law, probably dating back to the Napoleonic concept of university, provides no proper legal status . . .**

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trade unions, such as the Syndicat National de Chercheurs Scientifiques, for temporary research workers to be eligible for unemployment insurance. This was not conceded by the government, but a number of temporary postdocs were converted into a hybrid species, *fonctionnaires hors statut* (literally, civil servants outside the law), and as such attained tenure. DGRST found itself obliged to meet the cost of these new *fonctionnaires* and this limited its resources for maintaining posts for those who were still temporary, let alone creating new posts. For this reason, among others, scientific unemployment grew further. The unions became very restive and successfully prevailed upon DGRST to terminate its long established *contrats de recherche* (broadly similar to SRC research grants) which often incorporated money to pay for postdocs. The main aim was to force government agencies to make all research posts permanent, but the government has done the opposite. It has reduced the number of temporary posts without increasing the number of permanent positions.

Before this happened, industrial concerns had been in the habit of supporting contract research in universities, and financed a number of academic postdocs; but anxiety among young scientists facing the possibility of eventual unemployment without

state unemployment relief as well as union hostility, caused the withdrawal of industrial contracts, and these do not now appear to be a significant source of funds.

An unfortunate quirk of French law, probably dating back to the Napoleonic concept of the university, which provides no proper legal status and thus no insurance for those who are neither civil servants, self-employed nor employees of firms in the private sector, thus has seriously added to the plight of untenured French research workers. Initiatives to set up an unemployment scheme independent of the state do not appear, up to the present, to have met with success.

DGRST, aided by the new science minister, has striven valiantly to fill the gap left by the atrophy of contract research. In addition to some 3,000 2-year studentships for predoctoral students, and some 60 fellowships for tenured academics to enable them to devote some years to fulltime research, DGRST has now begun to provide postdoctoral fellowships available for allocation in the principal research laboratories. But at the time of writing the number available through DGRST is only of the order of 100, which falls a long way short of meeting the need. The greatest hardship is among newly graduated PhD's.

An influential working group of academics, industrialists and administrators presided over by Professor Jacques Friedel has been taking steps to assemble funds for some university fellowships, which have begun to be available at Orsay, Marseille, Strasbourg, Poitiers and Lille; other fellowships have been established in the State laboratories at Saclay and Grenoble. The insurance deadlock has not been resolved, but at least the decay of contracts is beginning to be circumvented.

This situation is however exacerbated by another specifically French problem, the firmly established tradition of young scientists to remain in the department where they researched their theses. Several administrators expressed the view to the author that the difficulty in persuading young scientists to move to other laboratories or to neighbouring disciplines, is the greatest single obstacle, after the insurance laws, to fuller scientific employment; and from a purely scientific standpoint, immobility has the grave disadvantage that the inflow of new ideas and experience into long-established laboratories is prevented.

R. W. Cahn

*The author is Professor of Material Sciences at Sussex University.*



# Snail-paced parasite that is marching through South America

Schistosomiasis, otherwise known as Bilharzia, is a debilitating disease causing anaemia, diarrhoea, abdominal pain and sometimes death. Its spread, initially through a particular species of freshwater snail, is now being accelerated by human carriers—Brazil's millions of migrant workers

Report by **David Bousfield** of Sussex University

UNTIL the 1920s schistosomiasis, a debilitating parasitic disease caused by *Schistosoma mansoni* which is transmitted by certain species of freshwater snail, was more or less restricted to the north-east of Brazil—an area which includes the states of Ceará, Rio Grande do Norte, Paraíba, Pernambuco, Alagoas and Sergipe. Then, as now, sugar cane cultivation along the coastal 'zona da mata' was a major source of employment. The sugar mills or 'engenhos' were invariably associated with river valleys providing alluvial fertilisation during the winter, water for irrigation, and ideal habitats for *Biomphalaria glabrata*, the main intermediate host of schistosomiasis in the area, which is capable of adapting to almost any kind of freshwater environment. Inland the climate becomes progressively drier and the corresponding vegetational zones, the 'agreste' and the 'sertão', supported simple farming and cattle ranching. In these semi-arid regions limited and intermittent sources of water meant concentration of population, pollution and disease and provided an environment for an intermediate host, *Biomphalaria straminea*, that was well adapted to temporary desiccation. Today, estimates for the levels of infection in Sergipe and Alagoas are above 30%, and even in the most developed state, Pernambuco, the level was almost 16% before 1975.

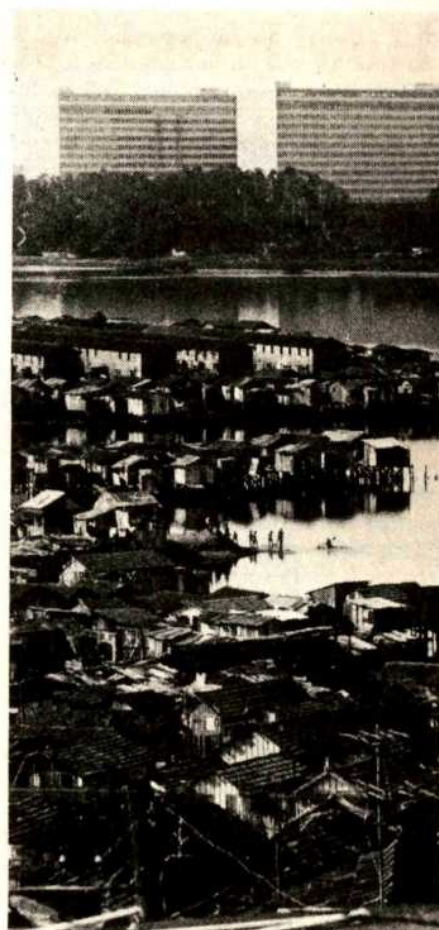
Unfortunately the disease did not remain in the north-east. Competition from the more progressive plantation owners in the south closed many 'engenhos' and forced others to adopt more mechanised, less labour intensive methods, whilst in the interior the severe droughts of 1958 and 1970, together with the 'strong-arm' tactics of the richer landowners forced many 'nordestinos' off their land and on to the road. These migrants travelled mainly to the south in search of work, taking their diseases with them. During the 1970 drought, for example, out of the total population of 30 million, 2 million people left the north-east. In 1937 schistosomiasis reached the state of Minas Gerais, and the states of Rio de Janeiro and São Paulo in 1950. In 1953

the disease reached Paraná bordering with Argentina and Paraguay, and it was recorded in Brasília in 1973. Since potential intermediate hosts were already to be found in all of these states it is presumed that human migration was responsible for the spread of the disease. By 1975 it was estimated that there were between 8 million and 18 million carriers and the disease cost \$150 million per year.

Human migration, however, is not the only reason for the rapid increase in size of the endemic area. Until about two decades ago only *Biomphalaria glabrata* and *B. straminea* were recognised as being the important intermediate hosts for schistosomiasis in South America. A third species *B. tenagophila*, found only in the south of the country (but whose distribution stretches westward and southward into neighbouring Bolivia, Paraguay, Argentina and Uruguay) was thought to have a very low susceptibility to infection by the miracidia of *S. mansoni*. Indeed in 1923 the Brazilian taxonomist Adolpho Lutz had proposed that the species be called *Planorbis immunis*. In 1956, however, Lygia Corrêa and others found a number of foci in the Paraíba valley which were maintained solely by *B. tenagophila*—in some areas snail infection rates were as high as 48%. Subsequent laboratory work conducted by Lobato Paraense and his co-workers have shown that *S. mansoni* is gradually adapting to its new snail host and that in the case of the strain from the Paraíba valley this process is particularly well advanced.

## Will the strain spread to Amazonia?

In a recent paper by Paraense and Corrêa they remark that "the susceptibility of most *B. tenagophila* populations to the S. J. [Paraíba] strain points to a fact of practical significance—the possibility of expansion of that strain to a wide South American area, most of which is still free of schistosomiasis". They also report that the strain is gradually spreading, and that this rate will increase as stronger migration incentives and better economic opportunities are developed in the region.



Brazil's waterside shanty towns: a focus for transmission of disease

In addition to this movement south, spread of schistosomiasis has been recorded, albeit on a much smaller scale, in the northern states of Maranhão and Pará. The intermediate host here is *B. straminea* but a new potential host *B. amazonica* has recently been discovered to the west with a distribution stretching from Porto Velho, along the Rio Solimões to Manaus. The possibility of spread to Amazonia is generally acknowledged amongst public health workers to be small. This judgement is based on the low population densities and high flow volumes involved, and the unsuitability of the water chemistry. However, the development of the Transamazonian Highway, built specifically to act as a 'safety valve' for the drought-plagued northeast, must challenge some of these assumptions. The original project, announced in 1970, called for settlement of 100,000 families along the pioneer highway by 1976—although, in fact, by September 1977 only 5,333 had actually been settled. Small towns were constructed every 10 km (agrovilas) with larger administrative and manufacturing centres built at 100 and 400 km intervals. Research conducted by Dr Nigel Smith (INPA, Manaus), however, has shown that as of September 1974 only 4 out of 26 agrovilas were equipped with piped water and



only 10 out of 26 possessed privies. Gastrointestinal problems due to the use of water from streams and ponds contaminated by human and animal faeces are already one of the major reasons for hospital admission. SUCAM, the government organisation responsible for disease control, maintains surveillance at Marabá, Altamira and in Rondônia, but to date no transmission has been reported, although many of the settlers are infected. However, at SUCAM's headquarters in Brasília, Dr Solon Camargo pointed out to me that agricultural activity and other attempts to modify forest ecology might produce favourable snail habitats in the vicinity of the highway. According to Dr Smith the 'slash and burn' techniques of the settlers and the application of lime to the soil has produced a large modification in the chemistry of stream and pond water close to the road and at a number of sites, notably Altamira *B. straminea* populations already thrive.

Until 1975 attempts to control Brazilian schistosomiasis took the form of small pilot projects usually aimed at reduction or eradication of snail populations using molluscicides. Some attempts to reduce the incidence of the disease by chemotherapy had also been made but neither strategy had been particularly useful in controlling transmission—small residual populations of infected snails could still maintain high rates of transmission and treated patients soon became reinfected, although it might be several years before disease reached its more advanced forms again. Indeed by this time many Brazilians working in schistosomiasis control felt that attempts to reduce the disease should be linked to community health programmes, and in particular to the provision of piped water, toilets and communal laundries. In rural Brazil

75% of households were without any form of toilet and 71% without protected water supplies in 1975. Dr F. S. Barbosa, a specialist in snail control wrote in 1974 that "attempts to control schistosomiasis, in a large scale, by using the conventional single control methods are futile and, more than this, may become detrimental to the developing communities."

### Better sanitation key to prevention

When the first national plan to control schistosomiasis (PECE) was announced in 1975 at the beginning of the term of office of President Geisel it was greeted by considerable criticism from the scientific community, for the Minister of Health, Dr Paulo de Almeida Machado proposed the eliminate, or at least control, the disease by mass chemotherapy—the drug oxamniquine (Mansil) being used most extensively. This choice of strategy was based on the success of a small pilot scheme in Paraná and the success of of an earlier mass injection scheme in which 80 million people were vaccinated against meningitis in 10 months. It is probable too that the need for positive results before the next election in 1978 played a part in the design of the programme. PECE began with two closely monitored experimental projects—one at the town of Tourós (population 2,250) in the state of Rio Grande de Norte, and the other at Santo Antonio das Trempes (pop. 571) in Pernambuco. At Tourós almost all the population was treated 'en masse' with oxamniquine during December 1975. Infection levels dropped from 56% to 5% but rose to 19% after one year. Indeed, for children under 15 years, the highest risk group, more than 50% became re-infected during this period. On the other hand, at Santo

Antonio das Trempes where chemotherapy was preceded by mollusciciding and installation of piped water, toilets, showers and communal laundries, the infection rate, which originally had been 50.4%, was still only 2% twenty months later. PECE has since been extended to cover seven north-eastern states, yet still relies heavily on drug therapy. Oxamniquine will be given to 12 million people (already between 1 and 2 million have been treated) whereas provision of sanitation is planned for only 2.6 million.

Additional programmes designed to control and stem the spread of schistosomiasis in the south are being undertaken by the São Paulo Secretariat of Health and in the neighbouring state of Paraná by SUCAM. São Paulo's problems began with its rapid industrial and agricultural growth with the concomitant need for manpower, irrigation systems and power stations. For example the state is now the country's biggest producer of sugar cane, a crop whose importance to the national economy will increase rapidly as the government's programme to substitute ethanol, produced from sugar, for gasoline gets under way. The need to reduce energy imports has also made hydroelectric power an increasingly attractive alternative and new schemes, such as the one at Itaipú, now have catchment areas extending into Argentina and Paraguay, making international transfer of schistosome strains a possibility. During the period 1967-70, the state received 113,054 migrants, mostly from the north-east and Minas Gerais. Routine coprological examination showed that an average 23% had schistosomiasis. By 1974 it was estimated that a quarter of a million infected migrants had entered the state. Attempts have been made to screen and treat infected migrants as they arrive. However, in order to prevent the spread of schistosomiasis such a scheme required that all migrants register with the local authorities, and that diagnosis and treatment are 100% effective. To date none of these standards has been achieved and the number of active foci within the state increases.

Whatever else happens in the future, it is clear that Brazil's need for cheap migratory workforces will continue to increase, and it is to be hoped that the new Minister of Health, Dr Mario Augusto de Castro Lima, with an extended 6 year term of office in front of him, will realise that chemotherapy is no match for the powerful social and economic forces which have made schistosomiasis a major problem in Brazil's agro-industrial age. □



Migrant sugar workers: attempts to screen them medically have failed

David Bousfield visited Brazil on a Nature Writing Fellowship



# Which researcher will get the grant?

*Jonathan R. Cole and Stephen Cole describe how disagreements among peer reviewers can explain the unexpectedly low correlation between a scientist's prior professional performance and his or her chances of being awarded a research grant.*

In recent years the system used by the United States government to distribute basic research funds for scientists has been under increasing attack both from members of the scientific community and from the American Congress. The most important criticism that has been raised is that the current system based upon peer review leads to inequitable decisions favouring eminent scientists and putting non-eminent scientists at a serious disadvantage in the competition for increasingly scarce funds.

For the past three years we have been conducting a detailed study of how the National Science Foundation decides whether or not to approve applications for research funds (Stephen Cole, Leonard C. Rubin, Jonathan R. Cole, *Peer Review in the National Science Foundation* (Washington: National Academy of Sciences, 1978)). We have done this work as consultants to the Committee on Science and Public Policy (COSPUP) of the National Academy of Sciences. Our study is based upon extensive qualitative interviews with people involved in all aspects of peer review, particularly NSF programme directors; an analysis of the actual reviews given to 250 research proposals; and a quantitative analysis of 1,200 applicants to 10 different NSF programmes in fiscal year 1975. The purpose of the quantitative study was to identify those characteristics of scientists and their 'track records' that were correlated with high or low peer ratings and with the final funding decision.

## 'Old boys club' theory disproved

Although the NSF peer review system had been criticised as being an 'old boys' club', our research did not support the contention that the methods used by the NSF in deciding who should receive funding were inequitable. We found that eminent reviewers did not favour applications from their eminent colleagues. Furthermore we found weak correlations between measures of the scientific performance of the applicant and the peer review ratings they received. Finally we found that the peer review ratings did in fact almost completely determine whether or not a grant was funded or declined funding.



One important result of the first phase of our finding of the first phase of the research concerned the relationship of past 'track record' of the applicant to the peer review ratings they received. For example, we found that the number of papers published in the last 10 years and citations to those papers had only a slight influence on ratings given to a proposal by the peer reviewers. In two of the 10 programmes we studied the proportion of variance explained on ratings by citations were 0.16 and 0.14, and in the other programmes less than 0.10. Other productivity or output measures are even less strongly related to the ratings.

We also found that the prestige rank of the applicant's current academic department had only a small to moderate correlation with the ratings given to their proposals. The criticism that applicants from prestigious East and West coast universities have a significantly better chance of receiving grants than those from other universities turned out to be unjustified.

In this discussion our primary concern is why we did not find the expected relationship between measures of the scientist's past performance, his position in the stratification system of science, and the peer review ratings received on his proposal.

One reason why the characteristics of applicants showed such low correlation with ratings received was that there was an unexpectedly large amount of disagreement among reviewers of the same proposal. Some reviewers gave proposals high ratings and other reviewers gave the same proposal low ratings. If the same applicant gets high ratings from some reviewers and low ratings from others, the correlation between any characteristic of the applicant and the ratings must necessarily be low.

In order to estimate the level of

agreement among reviewers of particular proposals we performed a one-way analysis of variance for each of the 10 programmes. In this analysis, the dependent variable was the rating that a proposal received from a reviewer; the independent variable was simply the proposal number, which is of course a nominal variable. The results presented in the accompanying table (overleaf) show the proportion of the total sum of squares for ratings on all proposals that is attributable to disagreements among reviewers of the same proposal.

## Lack of consensus is common to all subjects

Take, for example, the algebra programme at the NSF. If we examine all the variation that exists among raters of all proposals to the algebra programme, we find that 45% of the total variation is due to disagreements about the quality of individual proposals among the peer reviewers of the same proposal. The level of disagreement among reviewers of algebra proposals was by no means anomalous. We should also point out that contrary to our expectations there was no less consensus in the social sciences such as anthropology and economics than there was in the natural sciences. In fact, economics was the programme which showed the lowest proportion of variance (35%) explained by within-proposal variations.

These disagreements could be the product of several processes. First, there is substantially more actual cognitive disagreement among reviewers about the merits of proposed work by the applicant than is often believed to be the case. Second, the high amount of disagreement among reviewers could be in part a result of intersubjectivity, that is, two scientists might actually



Consensus among reviewers and influence of applicant characteristics on peer review ratings

	Lack of consensus among reviewers		Pearsonian correlation coefficients between				
	Percentage of total variance due to within-proposal variance	Log of citations to recent work and individual ratings	Log of citations to recent work and mean ratings	Rank of dept and individual ratings	Rank of dept and mean ratings	Combination of 9 characteristics and individual ratings*	Combination of 9 characteristics and mean ratings*
Algebra	45	.06	.11	.07	.10	.17	.31
Anthropology	43	.00	.01	.00	.00	.04	.07
Biochemistry	49	.16	.27	.07	.12	.20	.40
Chemical dynamics	45	.14	.25	.02	.04	.16	.29
Ecology	54	.01	.02	.02	.04	.06	.13
Economics	35	.08	.08	.13	.23	.21	.32
Fluid mechanics	43	.03	.02	.10	.10	.17	.29
Geophysics	57	.07	.14	.03	.07	.09	.21
Meteorology	63	.08	.16	.05	.11	.14	.37
Solid-state physics	55	.08	.16	.08	.16	.17	.38

\* The nine characteristics include: professional age, rank of Ph.D. department, rank of current department, academic rank, log of papers published in last 10 years, log of citations to work published in last 10 years, log of citations to papers more than 10 years old, whether institution is a Ph.D.-granting one or other, number of years funded by NSF in last five years.

have the same substantive appraisal of a proposal but may use different subjective scales of evaluation. Consequently, a similar evaluation can result in different actual ratings. Third, the disagreements could reflect some personal bias that the reviewers have against a particular principal investigator who submits a proposal. We could catalogue other potential sources of variations in the ratings, but the important, albeit tentative conclusion to underline is that the amount of variation in peer review ratings that could possibly be explained by characteristics of scientists and their track records is distinctly limited by this disagreement among reviewers over the same programme.

## Taking published papers into account

In order to eliminate the effects of this lack of consensus on the correlation between applicants' characteristics and ratings, we used as the dependent variable the mean rating received. As independent variables we used the log of citations to papers published during the last ten years prior to application, rank of the applicant's academic department, and a combination of nine characteristics measuring the scientist's location in the stratification system of science. The results of these regression analyses appear in the table.

Given that about one half of the variance in ratings is the result of within-proposal disagreement, the correlation between the characteristics of the applicant and the mean rating is about double the correlation between characteristics of the applicant and the individual rating. Although the correlations between individual characteristics and mean ratings are higher, they are still not as high as one might expect, particularly in fields such as solid state physics, algebra, or chemical

dynamics.

There are three reasons why these correlations are not higher. The first we call *self-selection*. Not everyone who is eligible to apply for an NSF grant does so. The group of scientists that applies for NSF research money tends to be a self-selected group of the most creative and productive scientists in their particular fields. In order to apply for a grant, an applicant must write a proposal that he or she knows will be sent out for review to colleagues in the discipline. Scientists who do not have active, ongoing research programmes are less likely to write such a proposal and subject themselves to peer review than those who do have active research programmes. Data in which we compare the productivity of the applicants to the NSF with productivity of random samples of academic scientists indicate that NSF applicants are on the whole a more productive group than are the population of scientists in a given field. Because self-selection takes place, the group of applicants from whom the reviewers must select successful proposals tends to have attenuated variance on some of the independent variables.

One reason for the low correlation between the rank of an applicant's academic department and peer review ratings is the relatively wide distribution of academic talent among American universities. Although it is true that, on the average, highly prestigious departments have more talented and productive scientists, a significant proportion of talented scientists are not located at the most prestigious departments. Several independent studies have found that the correlation between citations to a scientists' work and the prestige rank of his department is 0.30 or less. This suggests that quite a few scientists who have produced work judged to be useful and of high quality are not in highly ranked departments.

When we relate the low correlation between the quality of an individual scientist's research output and the rank of his department to the concept of self-selection, we can understand better the low correlation between the rank of an applicant's department and peer review ratings. If every scientist in every department applied for a grant, there would probably be a considerably higher correlation between rank of department and rating. But we know that all scientists do not apply. Applying scientists from low-ranked departments are probably the most active researchers of those departments. Whereas six mathematicians from MIT may apply for NSF funds in a given year, perhaps only one mathematician at a lower-ranked department will apply. But this one individual may have a national reputation comparable to those of some of his colleagues at high-ranked departments.

## Quality of science is main influence

Finally, the third and perhaps most important reason for the low correlation between characteristics of the applicants and peer review ratings is that all evidence collected thus far in the peer review study suggests that the ratings are strongly influenced by the reviewer's perception of the quality of the science contained in the proposal. If this is indeed true and the correlations between the ratings and the characteristics of applicants are low to moderate, this would necessarily mean that the correlation between characteristics of applicants and the perceived quality of their proposals would be low to moderate. We are currently analysing a set of data which will allow us to determine the extent to which this hypothesis is correct and the meaning of these data for peer review and the distribution of federal funds to research scientists. □

# news and views

## Temperatures in the clouds of Venus

from P. J. Houghton

ON board the Pioneer Venus orbiter which encountered the planet Venus on 4 December 1978 was a multi-channel infrared radiometer jointly built by the Jet Propulsion Laboratory, Pasadena, California and the Department of Atmospheric Physics at the University of Oxford. The Oxford contribution to this radiometer is the first British built experiment to leave the vicinity of the Earth and travel to one of the planets. Remote sounding observations with this radiometer have already provided the first information on the temperature structure in the upper Venus atmosphere (Taylor *et al.* *Science* **203**, 779; 1979). On page 613 of this issue of *Nature* Taylor and his colleagues at the Jet Propulsion Laboratory report some interesting new measurements of the detailed thermal structure of the clouds near the Venus pole which provide important clues to the nature of the circulation of the Venus atmosphere. Investigations from Earth-based telescopes and from spacecraft have demonstrated the very deep, rather uniform cloud cover on Venus, which completely obscures the surface from view. That the cloud droplets mainly consist of sulphuric acid was discovered as a result of the interpretation by Hansen, Arking and Young in 1973 of polarisation observations made at different phases of Venus by the French astronomers Coffeen and Gehrels in 1969. Although the size of the particles, as also deduced from these measurements, turned out to be a few microns only, the question remained as to how the upward motion necessary to maintain the clouds was generated, even though for such small particles the amount of upward motion need only be very small. A further question concerned the location of the compensating regions of downward motion. This week's report gives support to the theory put forward by Murray *et al.* (*J. geophys. Res.* **68**, 4813; 1963) and Suomi and Limaye (*Science* **201**, 1009; 1978) that intense regions of downward motion might be concentrated near the poles. In these regions clearing of the clouds might be expected, which would

not be visible from Earth because the polar regions are viewed so obliquely.

The Pioneer 12 orbiter, because it goes nearly over the Venus pole, has provided the first opportunity to observe the polar regions in detail. The infrared radiometer observations clearly show a localised region of much higher temperature than the rest of the cloud cover which has been interpreted as a local clearing of at least the upper half of the cloud layer. The measurements do not rule out a clearing to much deeper levels.

Observations from the infrared radiometer at altitudes of 60–90 km in the Venus atmosphere, well above the main cloud deck, have shown a temperature contrast of about 20 K between equator and pole, with the pole being warmer (Taylor *et al. op. cit.*). The obvious interpretation of these measurements in terms of circulation is of a Hadley type circulation with rising air over the equator, cooling as it rises, and sinking air around the polar regions, warming as it sinks. This is not unlike the thermally driven circulation which occurs in the mesosphere of the Earth's atmosphere where very cold temperatures occur at the mesopause (~85 km altitude) in summer contrasted with much warmer temperatures near the winter mesopause in the other hemisphere. Another important feature of the circulation at those levels above the main cloud deck is the very rapid zonal motion which occurs. Air moves around the planet at a velocity of about  $100 \text{ ms}^{-1}$ , one rotation of the planet taking about 4 days. This rapid motion ensures that there is little temperature contrast between the day and night side of the planet, as is confirmed by the Pioneer radiometer observations.

The solid surface of Venus rotates only very slowly—once in 243 days—so that the comparatively rapid rotation of higher parts of the Venus atmosphere is particularly intriguing. Schubert and Whitehead in 1969 (*Science* **153**, 71) put forward a possible theory of the mechanisms driving this circulation; they suggested it was in-

duced by a travelling thermal wave induced by the motion of the Sun relative to the atmosphere.

At altitudes above about 90 km in the Venus ionosphere, the thermal contrast between equator and pole largely disappears. Instead at these levels, as is shown again from infrared radiometer measurements, a temperature difference of about 20 K exists between the day and night side of the atmosphere, indicating a mean circulation which is primarily occurring between the warmer sunlit side and the cooler dark side.

It will be interesting to discover, from further work on the data from the infrared radiometer and from the other instruments on the Pioneer 12 orbiter, whether there is any evidence for large scale waves in the atmosphere above the Venus clouds of a similar or different nature to the planetary waves observed in the Earth's atmosphere. A big question which clearly arises is: how are the circulations in different parts of the Venus atmosphere linked? Is the upper atmosphere circulation, for instance, driven from below, or is it driven by heat sources and sinks near the cloud tops? A particularly interesting observation is that the cloud clearing reported in this issue is not actually at the pole but some  $10^\circ$  from it, suggesting that the circulation may have some interesting asymmetrical properties. Clearly a lot remains to be understood that will not only encourage those working on Venus data to extract from it information which will provide clues to some of the intriguing problems of the Venus circulation, but also provide the theoretical modellers with a challenge to fit it all together. As we begin to understand how the motions are organised in the Venus atmosphere, which is so different from our own, we shall in turn acquire deeper insight into what goes on in the atmosphere of the Earth. □

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# Neoplasias and B-cell precursors

from Maxime Seligmann

THE earliest recognisable cells within the B-cell differentiation pathway are known as pre-B cells. They are found in the fetal liver of rabbits, mice and men and are characterised by the presence of intracytoplasmic IgM and the lack of surface receptors<sup>1-3</sup>. In adults, pre-B cells are found almost exclusively in the bone marrow<sup>4</sup>. IgM can be detected by immunofluorescence techniques in the cytoplasm of most, if not all, large rapidly dividing pre-B cells and their small non-dividing pre-B cell progeny<sup>5</sup>, with an unusual perinuclear and reticular staining pattern. Pre-B cells are the progenitors of surface Ig positive B lymphocytes but, because they do not express functional immunoglobulin receptors on their surface, it can safely be assumed that antigens do not influence the diversity of immunoglobulins produced by pre-B cells. Various studies suggest that clonal diversification is accomplished by the pre-B cell stage. For example, individual pre-B cells show allelic exclusion in rabbits heterozygous for kappa chain allotypes<sup>6</sup>. In myeloma patients, antibodies specific for idiotypic determinants of the myeloma globulin stain bone marrow cells with pre-B cell characteristics<sup>7</sup>; this finding provides evidence that pre-B cells belong to the neoplastic clone and suggests that V-C joining has occurred at this stage.

Several recent studies have shown that some murine and human neoplasias involve such early B-cell precursors which produce cytoplasmic immunoglobulin in the absence of membrane-bound immunoglobulin molecules. The Abelson murine leukaemia virus complex, which consists of a replication-defective virus and its helper Moloney leukaemia virus, causes thymus-independent lymphomas in Balb/c mice, and several characteristics suggested that these tumours were related to the B-cell lineage<sup>8</sup>. Two independent studies<sup>9,10</sup> have recently provided evidence that several neoplastic cell lines resulting from *in vivo* or *in vitro* transformation by Abelson virus have features indicative of early maturation compartments in the B-lymphocyte lineage. On the other hand, two human lymphoid cell lines (Raji and T51) were also shown to express characteristics of early B-cell precursors<sup>11</sup>. Finally the

leukaemic cells of some patients with acute lymphoblastic leukaemia also display features thought to be characteristic of pre-B cells. This pre-B cell phenotype is not uncommon because it was found in four of 22 patients in the initial report by Vogler *et al.*<sup>12</sup> and in 10 of 68 patients in the subsequent series of Brouet *et al.*<sup>13</sup>.

The results of immunofluorescence and immunoglobulin synthesis studies suggest that the maturation arrest in these various neoplastic cells may occur at slightly different steps in the early stages of the B-cell pathway. Early studies indicated that normal pre-B cells produce complete monomeric IgM molecules with both  $\mu$  and light chain<sup>1,2</sup>. In contrast, no light chain determinants could be detected by immunofluorescence in most human leukaemic pre-B cells<sup>13</sup>. This negative finding could be due to limitations of immunofluorescence technology because, in the human cultured cell lines mentioned above, light chains could not be detected by immunofluorescence but were found at the ultrastructural level by an immunoperoxidase method<sup>14</sup>. It should be noted, however, that the number of normal pre-B cells containing detectable light chain determinants is lower than the number of such cells with detectable  $\mu$  chains<sup>14</sup>. This discrepancy and the findings in acute lymphoblastic leukaemia are consistent with the possibility that there is an asynchronous onset of heavy and light chain synthesis. The data reported by Siden *et al.*<sup>10</sup> support this hypothesis because most Abelson-virus-induced cell lines synthesise a  $\mu$  chain but no

detectable light chain. This  $\mu$ -only phenotype was stable on subcloning. Cultures in which synthesis of both  $\mu$  and  $\kappa$  chains occurred showed a rapid loss of  $\kappa$  chain synthesis. One line was studied in detail: the intracytoplasmic molecules represented glycosylated  $\mu$  chain dimers which were not secreted. Light chain synthesis could not be induced by reducing the growth rate of the cells, a procedure that resulted in increased  $\mu$  chain synthesis. In contrast to these findings, another Abelson-virus-derived line studied by the same authors produced only  $\kappa$  chains. It should be emphasised that the biosynthesis experiments showed that the cytoplasmic extract contained, in addition to the  $\kappa$  chain band, a slower band which comigrated with a precursor polypeptide. It is interesting in this respect that the intracytoplasmic IgM of the human lines was not located inside endoplasmic reticulum but at the level of free polyribosomes<sup>11</sup> and that these lines produce abnormally large Ig polypeptide chains (unpublished results of P. Guglielmi and J. L. Preud'homme). The two Abelson-virus-transformed cell lines studied by Boss *et al.*<sup>9</sup> apparently synthesised monomeric IgM with both  $\mu$  and light chains. However, most cells in both lines were presumably less mature than pre-B cells because they were negative for cytoplasmic IgM but could be induced to express IgM positivity by several compounds such as lipopolysaccharide, DMSO and butyric acid. The human lymphoid cell lines are presumably slightly more differentiated than *bona fide* pre-B cells because they bear Ia-like determinants, they secrete polymeric IgM molecules (instead of monomeric IgM in normal pre-B cells<sup>2</sup>) and they become able to produce membrane-bound immunoglobulin molecules in certain conditions (unpublished results of P. Guglielmi and J. L. Preud'homme).

Another point of interest is that human pre-B leukaemic cells in a fair percentage of patients<sup>13</sup>, as well as several Abelson virus-induced tumours<sup>15</sup> and normal pre-B cells (G. Janossy, personal communications), were shown to contain low but detectable levels of terminal deoxynucleotidyl transferase. This enzyme was initially considered as characteristic for thymic and pre-thymic cells but it is probably present in progenitors of all lymphoid cells, a finding which might be related to the possible role of this enzyme in the generation of somatic mutations<sup>16</sup>.

The availability of tumour cells and continuous lines corresponding to early precursors of the B lineage lymphoid cells should be useful for the study of immunoglobulin gene diversification and expression. □

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## Hepatitis A: a giant leap forward

from Arie J. Zuckerman

THE past decade has seen rapid progress in the identification of viruses causing hepatitis in man, starting in 1968 with the discovery of the association between Australia antigen and hepatitis type B (serum hepatitis). Five years later, hepatitis A virus, which is the cause of infectious or epidemic hepatitis, was identified by electron microscopy in faecal extracts, and more recently evidence has been obtained of a third type of hepatitis (so called non-A, non-B hepatitis) which is probably caused by more than one virus. The worldwide research effort which has been devoted to the problem of viral hepatitis by epidemiologists, virologists, molecular biologists, immunologists, pathologists, clinicians, blood transfusion services, public health authorities and others reflects the impact which these viruses have on human health and welfare. Although remarkable advances have been made in the characterisation of the biophysical and biochemical properties of hepatitis A and B viruses, reproducible propagation of these viruses in tissue culture has defied every effort and continued to elude even the most stubborn research worker. Provost and Hilleman (*Proc. Soc. exp. Biol. Med.* **160**, 213; 1979) now report the first reliable serial cultivation of human hepatitis A virus in primary explant liver cell cultures of marmosets and in a fetal rhesus kidney cell line. This work represents a spectacular advance in a hitherto frustrating and seemingly insoluble problem.

The events which led to this success started in 1967 when Deinhardt and his associates (*J. exp. Med.* **125**, 673; 1967) transmitted human hepatitis A virus to two species of marmosets, which are small South American monkeys, and the infection was transferred serially from animal to animal. Differences in susceptibility to hepatitis A exist between the marmoset species, with *Saguinus mystax* being the most susceptible. In 1973, the CR 326 strain of hepatitis A virus was isolated in *S. mystax* by Mascoli *et al.* (*Proc. Soc. exp. Biol. Med.* **142**, 276; 1973) from naturally occurring outbreaks in Costa Rica, and the virus was further characterised in the sera and livers of

the marmosets (Provost *et al.* *Proc. Soc. exp. Biol. Med.* **148**, 532; 1975). By then sensitive serological tests for hepatitis A virus and its antibody became available and the susceptibility of chimpanzees as well as the susceptibility of a more readily available rufiventer-like marmoset designated as *S. labiatus* (also known as *Marikina labiata*, *Jacchus rufiventer*) was described (Provost *et al.* *Proc. Soc. exp. Biol. Med.* **155**, 283; 1977).

Using the CR 326 strain of human hepatitis A virus passaged five times in *S. mystax* and 26 times in *S. labiatus*, Provost and Hilleman inoculated normal liver explant cell cultures obtained from *S. labiatus* with the virus, and observed by direct immunofluorescence minute fluorescing cytoplasmic granules in some of the hepatocyte-like epithelial cell outgrowths as early as 8 days after inoculation. The number of the affected hepatocyte-like cells and the number of fluorescent granules increased with time so that when cultures were collected on day 23, 75–100 % of these cells, but not other cell types, contained large numbers of granules. There were no cytopathic changes. The virus was identified as hepatitis A by various techniques including specific immunofluorescence blocking, serum neutralisation, immune adherence haemagglutination and radioimmunoassay; and the virus was passaged serially five times. Because the availability of marmosets is very limited, an alternative cell culture system was sought. The virus propagated in primary kidney cell cultures of *Cercopithecus aethiops* (grivet or green monkey), but many of the cultures contained cytopathic adventitious agents of endogenous origin. Consequently, Provost and Hilleman used cultures of fetal rhesus kidney cells (designated FRhK6 cell line), which are epithelial-like but have only a maximum population doubling of 12. Specific immunofluorescence was detected in these cells as early as 3 days after inoculation and occurred throughout the cell sheets during the following weeks. Cytopathic changes were not observed. The virus was passaged serially, the incubation period for development of fluorescence was shortened, and there was a larger yield in these cells than in the liver cell explants. Titration of infectivity of the virus collected in the fifth and sixth passage yielded titres of  $10^6$ – $10^7$  infectious units per ml respectively, and virus in the fifth passage collection retained the ability to infect *S. labiatus* marmosets. The virus was successfully transferred through eight serial passages in which the calculated dilution of the original inoculum was  $2.9 \times 10^{-20}$ .

The importance and practical significance of this work can be summarised as follows: the means may now be available for detecting human hepatitis A virus *in vitro*, for preparing large quantities of viral antigen for serological tests and for providing a source of viral antigen for the preparation of vaccines against an important infection which is endemic in all parts of the world.

However, answers to several questions are still required. Can the virus be cultivated from naturally occurring strains without adaptation by serial passage through susceptible marmosets and what is the minimum period of adaptation required; can hepatitis A virus be propagated in more readily available cell lines with longer population doubling times? Provost and Hilleman provide a hint that this may be so because the virus replicated to a limited extent in human diploid lung fibroblasts, which are commonly used for the preparation of vaccines. The way may thus be open in the near future to the effective control of epidemic hepatitis. □

## Vacancies in nickel-aluminium and other alloys

from Robert W. Cahn

IN 1937 the metallurgist A. Taylor made a celebrated study of the structure of nickel-aluminium alloys; this called for great experimental precision, and in 1972 he returned to the problem and examined it with even greater scruple (Taylor and Doyle, *J. appl. Cryst.* **5**, 201; 1972). This second study may be said to have launched a new wave of research on the Ni–Al system and its analogues, even though few of the most recent protagonists seem to be aware of Taylor's second study.

Taylor's experiments centred on the alloy  $\beta$ -NiAl, which adopts the very simple B2 (CsCl-type) structure: nickel atoms at cube corners, aluminium at cube centres. Taylor measured macroscopic densities and lattice parameters as a function of composition, either side of the 50/50 composition: by putting together the two sets of measurements, he was able to deduce the destinations of excess nickel or aluminium atoms. On the nickel-rich side, the extra nickel atoms substitute in the usual way for aluminium atoms on the aluminium sublattice. The aluminium-rich side, however, is quite different: no aluminium substitutes on the nickel sublattice, instead nickel atoms disappear from the nickel sublattice, leaving nickel vacancies. For instance, at 45

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at. % Ni, 18 % of the nickel sites are vacant, all of the aluminium sites are filled. Such vacancy populations, determined by composition and not temperature, are distinguished as constitutional vacancies.

Several other studies showed that stoichiometric  $\beta$ -NiAl quenched from a high temperature (as opposed to that slowly cooled) contained a high concentration of thermal vacancies; the most recently cited figure is 1.08% of vacancies at 1,600 °C. This is a very much larger thermal vacancy concentration than is found in other metals or alloys, even just below the melting temperature; so large that on cooling the vacancies will separate out into a population of voids visible in the electron microscope (Epperson *et al. Phil. Mag.* **A38**, 529; 1978). 50/50 NiAl containing such vacancies, all on the nickel sublattice, must also contain substitutional defects—that is some nickel atoms in the aluminium sublattice, also called nickel antistructure atoms—to preserve the overall chemical composition: specifically, two vacancies must be accompanied by one substitutional defect. Such a trio of linked defects is now termed a triple defect and much recent research has centred on this entity.

In recent years, investigators in the US, England and Germany began to look for parallels to this curious behaviour in alloy systems isomorphous with  $\beta$ -NiAl, and attention soon centred on NiGa and CoGa. It became clear that these two phases always contain triple defects, even when they have been cooled slowly to eliminate thermal vacancies as far as possible. Though there has been some confusion in the literature on this point, it is now adequately clear that the triple defects in these alloys at 50/50 composition are indeed wholly of thermal, not constitutional origin; it is impossible to eliminate all thermal defects, even by very slow cooling.

A study of NiGa by Donaldson and Rawlings (*Acta Met.* **24**, 811; 1976) again made use of measured densities and lattice parameters to assess just how the concentration of nickel vacancies and gallium antistructure atoms varies with composition. To obtain these values, they developed an iterative method of calculation, starting out with an arbitrary assumption about the number of triple defects at stoichiometry, and refining this value in cycles until experimental densities and lattice parameters at a range of compositions could be matched. (To do this, it is necessary to assume a value for the relaxed volume of a nickel vacancy, which has to be based on theoretical

estimates, and comparison of some recent papers shows that this is the weak link in the chain of theoretical deduction.) Donaldson and Rawlings found just over 3% of vacancies and half that number of antistructure atoms in 50/50 NiGa. At 47% Ni, there are 7% vacancies and 0.2% antistructure atoms, while at 54% Ni, the defect numbers are 1% and 4.5% respectively. The triple defect itself (a 2/1 ratio of the two kinds of defect) only exists at stoichiometry. Ho *et al. (Scripta Met.* **11**, 1159; 1977) obtained rather similar values, though they do not match Donaldson's and Rawlings' figures in every particular.

Berner (dissertation, Stuttgart, 1976; Berner *et al. J. Phys. Chem. Solids* **36**, 221; 1975) has found closely similar values for defect densities in CoGa.

The computational problem of determining defect concentrations from experimental data when both defect types are present has now been eased by an ingenious application of statistical thermodynamics to the problem. Edelin (*Acta Met.* **27**, 455; 1979) calculated the relations between composition, defect concentrations and defect formation energies, using merely the hypothesis that the formation energies for Ga vacancies and antistructure atoms on the Ni (or Co) sublattice are so much higher than the energies for Ni (or Co) vacancies and antistructure atoms on the Ga sublattice that the former two defects never appear in detectable numbers. Edelin showed that the variation of concentration of the two defect types, both as a function of temperature and as a function of composition, was determined by a single composite activation energy,  $Q$ , and  $Q$  can be determined directly by fitting, for a range of compositions, experimental values of density and lattice parameter to his theoretical equations. Further, it appears that when fitting is done in this way, the relaxed volumes of vacancies and of antistructure atoms can be deduced from the measurements and do not need to be used as assumed input values. (This claim seems so remarkable that it deserves critical appraisal by a suitably qualified theoretician: it would seem to be the first claimed method for deductively obtaining relaxed defect volumes from experimental data.)

The claim by Edelin to be able to measure the relaxed volumes of defect sites is of particular interest because it has been established, from diffuse X-ray scattering measurements made at Stuttgart, that the lattice near a vacancy in NiAl or CoGa is severely distorted in a [111] direction. This distortion is of the same type as is found in a  $\beta \rightarrow \omega$  phase transformation. Such distortions must surely affect the relaxed vacancy volume (Ortiz and Ep-

person, *Scripta Met.* **13**, 237; 1979, for NiAl; Kirchgartner and Gerold, *J. appl. Cryst.* **11**, 153; 1978, for CoGa).

The composite activation energy,  $Q$ , found by Edelin for NiGa is 0.29–0.32 eV per atom and for CoGa, 0.265 eV per atom. These very low values account for the high thermal defect concentrations found even at stoichiometry in these two alloys. Edelin's method has not been used for NiAl (no accurate measurements of thermal defect concentrations at stoichiometry being available), but  $Q$  can be estimated from measurements of nickel diffusion in NiAl at a range of compositions (Hancock and McDonnell *Phys. Stat. Sol. A* **4**, 143; 1971). On the low-nickel side, it is assumed that the measured activation energy for diffusion is purely that for vacancy migration (because there is an abundance of constitutional vacancies available); then it is possible to compute the formation energy for thermal vacancies by subtracting the migration energy from the measured activation energy for diffusion at stoichiometry (where no constitutional vacancies exist). This approach gives 0.70 eV per atom, corresponding to the much smaller thermal vacancy concentration in this alloy. This much higher  $Q$  value also accounts, by way of Edelin's theory, for the virtual absence of aluminium antistructure atoms on the nickel-poor side in this alloy series, as contrasted with NiGa.

There have been several attempts to bypass the density/lattice parameter approach, which makes severe demands on experimental accuracy. (One difficult problem is to get precise densities in view of the presence of casting porosity in ingots; Taylor and Doyle overcame this by high-pressure annealing of ingots, and other investigators have sought to bypass it by using dilatometry instead of Archimedean weighing.) An alternative approach is to measure thermodynamic activities at high temperature and to relate such activities to defect concentrations. A theory to make this possible has been constructed by Neumann, Chang and Lee (*Acta Met.* **24**, 593; 1976) and has been applied to NiGa (Neumann *Scripta Met.* **11**, 969; 1977). The vacancy concentrations in NiGa alloys deduced from activity values are considerably lower than those obtained by the conventional method with quenched samples, but a very recent paper (Neumann and Chang *Z. Metallkde.* **70**, 118; 1979) shows that if allowance is made for loss of vacancies during quenching, then agreement is better. Nevertheless, it does seem that there must be some residual error in Neumann's statistical thermodynamics, and one possible source of such error is the tacit assumption that vacancies and antistructure atoms are randomly



disposed. Delavignette, Richel and Amelinckx (*Phys. Stat. Sol. A* **13**, 545; 1972) have adduced electron-microscopical evidence pointing to such order among the vacancies in hypostoichiometric NiAl, and Reynaud (*J. appl. Cryst.* **9**, 263; 1972) has evidence suggesting ordered antistructure atoms in hyperstoichiometric NiAl. Any short-range order among defects would have a considerable effect on thermodynamic activities. The recently observed anisotropic distortion around vacancies, already cited, with its associated stress field, could also affect activities. The activity method is therefore suspect, but it must be admitted that substantial order or lattice distortions could introduce errors into the density/lattice parameter method as well.

Surprisingly little attention has been paid to the question why  $\beta$ -NiAl, NiGa

and CoGa form defect structures at all. For instance, Massalski and Mizutani, in their survey of electronic structures of Hume-Rothery phases (*Prog. Mater. Sci.* **22**, parts 3/4; 1978) make no reference to these phases. Taylor and Doyle (*loc. cit.*) point out that the electron/atom ratio remains strictly constant at 3/2 from 50/50 NiAl to the phase boundary on the aluminium-rich side (where there are only 1.75 atoms per cell), and they assume this feature to govern the introduction of vacancies. The question then arises why other isomorphous alloy series such as Ni-Zn or Co-Be do not behave in this way. Machlin (*Scripta Met.* **13**, 123; 1979) has made an ingenious approach to this question by suggesting that the self-energy of the B (non-transition) component may be lowered by a reduction in the number of bonds between

B and transition metal atoms, resulting from the presence of transition metal vacancies adjacent to some B atoms. He is able to show that on this hypothesis, constitutional vacancies should be stabilised only for trivalent B metals, such as Al or Ga, and this is in accord with observation. There is scope for further theoretical treatment of the reasons for the formation of constitutional vacancies.

Clearly NiGa and CoGa in particular are ideally suited as test materials in which the predictions of precise statistical thermodynamics applied to defect populations can be compared with experimental observations. They are probably the best metallic materials for such comparisons, free of the complications arising from the need for charge neutrality which are unavoidable with ionic crystals. □

## Interferons and inducers *in vivo*

from Nowell Stebbing

MUCH is known about the mode of action of the interferons at the molecular level (Friedman *Bact. Rev.* **3**, 543; 1977), and intensive effort is being devoted to investigations of their structure and the cloning of the requisite genes. Although less attention has focused on the factors necessary for interferons to be effective in the treatment of virus infections, reports are now beginning to demonstrate the importance of host factors. The most recent such report (Shellekens *et al. Nature* **278**, 742; 1979) shows that interferon (from human leukocytes) is protective in monkeys against infection with a strain of vaccinia virus that does not respond to interferon in cultured cells. (The same cell lines are protected by the interferon against infection with vesicular stomatitis virus.) These results indicate that, *in vivo*, leukocyte interferon at least, has anti-viral effects by means other than the known molecular mechanisms. In view of the diversity of interferons in terms of immunological differences and cellular origin, it will be important to determine whether other interferons behave similarly, before these observations can be generalised. However, it is possible that the known molecular effects of interferons do not contribute directly to their anti-viral effects *in vivo* in any situation. Even when an interferon does inhibit virus replication in cell cultures, anti-viral effects in whole animals may be due entirely or in part to properties of particular cells of the host. Clearly, the anti-viral effects of interferons against infections of fibroblasts in culture should not be

extrapolated to treatments of whole animals, although this has been done for many years.

The issue of whether interferon inducers have anti-viral effects by means other than or additional to known molecular mechanisms has been highly contentious. Non-interferon-mediated anti-viral effects of polynucleotides have always been a possibility and claims that interferon induction is the sole mechanism of action have generally ignored the fact that such claims require quantification: it is necessary to know the extent to which observed anti-viral effects are due to interferon induction. It is now apparent that quantification by extrapolation from tissue culture studies is misleading. The most recent evidence in favour of interferon as the sole mediator of the anti-viral and anti-tumour effects of the double-stranded polynucleotide interferon inducer, poly(I):poly(C), is that of Gresser *et al. (Int. J. Cancer* **21**, 72; 1978). They demonstrated that anti-mouse interferon serum raised in sheep abolished the anti-viral and anti-tumour effects of poly(I):poly(C) administered to mice. However, mice treated with an anti-interferon serum, without simultaneous poly(I):poly(C) treatment, died earlier from virus infection than control mice and therefore the anti-serum may primarily affect virus- rather than poly(I):poly(C)-induced interferon. For such reasons it remains possible that poly(I):poly(C) has anti-viral and anti-tumour effects in addition to interferon induction.

Depletion of macrophages in mice obliterates the anti-viral effects of crude (macrophage) interferon and decreases the anti-viral effects of purified fibroblast interferon (Stebbing *et al. Inf.*

*Immun.* **19**, 5; 1978). Moreover, interferon and interferon inducers (Gidlund *et al. Nature* **273**, 759; 1978; Herberman *et al. Cancer Treatment Repts* **62**, 1893; 1978) are known to increase natural killer cell activity, implying that activities of these cells may be important for *in vivo* efficacy of interferons. Thus particular cell populations in whole animals seem to be decisive in determining the anti-viral and anti-tumour effects of interferons, and further work on the nature of their involvement would seem important for several reasons. It will of course be fascinating, to determine whether and in what ways the known molecular and cellular effects of interferons contribute to anti-viral and anti-tumour effects *in vivo*. In addition, clinical potential can only be determined by clinical studies whatever the theoretical arguments. However, it seems likely that the natural or induced immunosuppression associated with particular diseases could limit the efficacy of interferon therapy. The disappointment of negative results in clinical trials might be avoided by considering the state of cell populations necessary for *in vivo* efficacy of the interferon being tested. There is also the possibility of devising means of directly eliciting cell mediated anti-viral effects by short-circuiting the interferon mechanism.

Although interferon induction has generally been considered the means whereby polynucleotides have anti-viral and anti-tumour activity, other means are now apparent whereby even double-stranded polynucleotides could inhibit virus replication, for example by direct inhibition of protein synthesis (Levin & London *Proc. natn. Acad. Sci. U.S.A.* **75**, 1121; 1978; Kerr &

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Brown *Proc. natn. Acad. Sci. U.S.A.* **75**, 256; 1978) possibly by means of a nuclease which degrades viral mRNA (Baglioni *et al. Nature* **273**, 684; 1978). The last possibility provides a mechanism for interfering with virus replication in a manner related to that of interferons and their inducers but without actual production of interferon. The anti-viral effects of single-stranded polynucleotides which do not induce interferon may occur by this means or by mimicking strategic nucleotide sequences in viral RNA (Stebbing *et al. Ann. N.Y. Acad. Sci.* **284**, 682; 1977). It is noteworthy that early studies on interferon induction by polynucleotides involved largely single-stranded RNAs (Isaacs *et al. Lancet* **ii** 113; 1963; Rotem *et al. Nature* **197**, 564; 1963) and that two separate mechanisms may have been involved in the overall anti-viral effect obtained. Induction of interferon was certainly a factor but independent more direct anti-viral effects may also have been involved. □

## Flattening of Uranus and Neptune

from David W. Hughes

PLANETARY flattening or oblateness,  $\epsilon$ , is defined as the difference between the equatorial and polar radii divided by the equatorial radius. It is not an easy quantity to determine especially in the case of the outer planets. The flattening can be obtained geometrically by using for example a double image micrometer in the focal plane of a large telescope or by the analysis of the isophotes of the image of the planet obtained from spacecraft photographs. It can be measured dynamically by observing the orbital perturbations of the inner satellites of the planet induced by its unsymmetrical mass distribution, calculating  $J_2$ , the quadrupole moment of the gravitational field and using the simple relationship between this quantity and the flattening  $\epsilon$ . Unfortunately if we take the fairly typical case of Uranus, the inner satellites Miranda, Ariel and Umbriel form a system with near commensurability of mean motions, a state of affairs that results in important mutual perturbations.

A. H. Cook of the Cavendish Laboratory, University of Cambridge reviews recent observations of  $\epsilon$  in the light of the new determinations of the spin periods of the planets and shows that some of the values obtained for  $\epsilon$  are implausible to say the least. His conclusions are presented in a recent edition of the *Monthly Notices of the Royal Astronomical Society* (**187**, 39P;

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1979).

For a planet in hydrostatic equilibrium the product  $C/Ma^2$ , where  $C$  is the moment of inertia about the polar axis,  $M$  is the mass and  $a$  is the radius, must be between specific limits. This quantity is 0.4 if the planet is of uniform density and drops to 0 for an extreme central condensation.

Unfortunately the present day values obtained for the dynamical properties of Uranus and Neptune are confusingly diverse. For Uranus, recent measurements of the spin period give two inconsistent values, one about 24 h and the other about 14.5 h. There are two values for Neptune as well, one about 22 h and the other about 18.5 h. Inconsistent values have been found for one or both planets for  $\epsilon$  and  $J_2$ .

Cook then considers all possible combinations of the observed parameters to see which if any can be rejected due to the physical unreality of the value of  $C/Ma$  so produced. The central pressures of Uranus and Neptune are comparable with those of the Earth, however the mean molecular weights of the material in these planets are less and the material is more compressible. Cook concludes that these planets have  $C/Ma$  values which are definitely greater than 0.25 and probably in excess of 0.3. On this basis both the values obtained for the geometrical flattening of Uranus, 0.028 and 0.01, are inadmissible. It is not possible to decide between the three  $J_2$  values of 0.00343, 0.005 and 0.012. For Neptune the  $\epsilon$  and  $J_2$  values both support the lower spin period of 18.5 h.

To resolve this sorry state of affairs the astronomical community must be stimulated into taking new observations. Unfortunately, as is so often the case, the best place to take these observations is outside the distorting atmosphere of planet Earth. □

## More spots before the eyes

from E. G. Richards

TREATMENT of *Escherichia coli* DNA with *EcoRI* restriction enzyme results in an estimated 500–600 fragments of DNA differing first in their molecular size and second in their base composition and sequence. If such a digest is examined by electrophoresis in agarose, a technique which separates DNA according to molecular size, a relatively featureless pattern is obtained, for the resolving power of the method is insufficient to resolve such a large number of components. How useful it would be, however, if this were possible.

Fischer and Lerman (*Cell* **16**, 191;

1979) have recently described a two-dimensional electrophoretic method which approaches this ideal. They start with the observation that a mixture of urea and formamide—both non-ionic substances—can denature DNA. That is to say, if the concentration of these denaturants is increased, a point is reached at which the DNA wholly or completely 'melts'. This critical concentration depends on the temperature, occurring at a lower concentration the higher the temperature, and also on the base composition and sequence, albeit in a complicated way.

In their first experiment Fischer and Lerman applied a sample of an *EcoRI* digest of  $\lambda$  phage DNA, containing six fragments, along the top of a slab polyacrylamide gel and subjected it to electrophoresis downwards. They had, however, arranged that the gel contained a gradient of concentration of urea and formamide in a direction perpendicular to the direction of migration. After electrophoresis they observed that in the part of the gel where denaturant concentration was low the six fragments were resolved according to molecular size and moved with a velocity almost independent of denaturant concentration. On the other hand, on the high-concentration side of the gel the components moved with a velocity reduced by a factor of 20 or 30 and remained near the top of the gel. At intermediate parts of the gel where the denaturant concentration took intermediate values, the components moved with intermediate velocities. The result was that the zone of DNA corresponding to each fragment was distorted into a sigmoidal curve resembling a 'melting curve'. Corresponding to each fragment there was a critical denaturant concentration at which the mobility fell to a low value and at which partial or complete denaturation took place. This critical concentration varied from one fragment to another and no doubt depended on the composition and sequence of the fragment. If the temperature at which the gel was maintained was reduced (from 60 °C to 45 °C) this critical concentration increased as expected. It may be noted that this simple description was slightly complicated for one or two fragments by the presence of more than one step in the 'melting curve', a phenomenon probably associated with the denaturation of separate domains of the DNA at different denaturant concentrations.

These observations are certainly entertaining but not of great apparent importance. This importance, however, is provided in the next and crucial stage of Fischer and Lerman's work when they rotated the denaturant gradient through 90° so that the concentration of urea and formamide increased in the direction of migration. They then ob-

served that each fragment set out on its passage down the gel with a velocity corresponding to its mobility in the native state. Sooner or later, however, each fragment reached a point in the gel at which it denatured and its mobility was reduced to a low value. At that point it came (almost) to a halt and was, as an added bonus, sharpened. A fragment came to a halt at a point which was not correlated with its size so that zones which were initially faster might be overtaken by larger and slower fragments if these were the more stable. The result was that the fragments were sorted out into the order of their stability rather than of their size.

The final step was then to perform an initial separation of the fragments in agarose to obtain a separation on the basis of size and then to lay a strip of the resulting agarose gel on top of a slab polyacrylamide gel and subject the separated fragments to electrophoresis in a direction perpendicular to the first and into a denaturant gradient. The result was six well separated spots distributed over the area of the slab. That is to say they were distributed in the two-dimensional electropherogram according to molecular size in one direction and according to stability in the other.

They then applied the method to an *EcoRI* digest of *E. coli* DNA and resolved up to 350 discernible spots. The authors suggest that further improvements in the sensitivity of detection might result in the appearance of more spots and point out that their method is capable of considerable further refinement. Meanwhile the significance of the method is demonstrated by the detection of four spots unique to a digest of lysogenic  $\lambda$  lysogen of *E. coli* which were not observed in uninfected *E. coli* DNA. Two of these corresponded in position to the spots obtained with  $\lambda$  phage DNA alone.

Meanwhile a rather similar trick was used by Creighton (*J. molec. Biol.* **128**, 235; 1979) in a study of protein denaturation. He subjected various proteins to electrophoresis perpendicular to a urea gradient in a slab polyacrylamide gel. He observed that although the mobility of these changed slowly as the concentration of denaturant was increased, possibly due to the effect of the urea on the viscosity of the interstitial fluid in which the proteins migrated, they underwent a more pronounced decrease in mobility at a critical concentration of denaturant at which denaturation took place. Creighton gives a mathematical appendix in which he analyses the distribution of protein in these sigmoid shaped zones

in terms of the mobilities and rates of denaturation and renaturation of the protein. Using this technique, he obtains further evidence of the all or none character of the denaturation of several proteins and in others of more complicated occurrences.

These two papers demonstrate again the remarkable versatility of gel electrophoresis without which life would be much less exciting to molecular biologists. It makes me wonder what the next jelly trick will be.  $\square$

## Pattern formation in chick limb bud

from Jonathan Slack

SOME biologists believe that a problem is as good as solved as soon as the right animal has been found with which to work, and in research on biological pattern formation the limb buds of the chick embryo have become a favourite system for study. In an organ such as this it is immediately clear that spatial patterning presents a problem separate from that of cell differentiation. This is because the limb consists of only a few cell types (muscle, cartilage, bone and connective tissue) but is built up of a complex quasi-periodic arrangement of these components in three dimensions, and the mechanism which brings about this arrangement demands explanation. A glimpse at the difficulties involved is provided however, by two recent papers in the *Journal of Embryology and Experimental Morphology*, which report attempts to test two of the most influential recent models (Wolpert, Tickle and Stampford *J. Embryol. exp. Morph.* **50**, 175; 1979; Summerbell *ibid.* 217; 1979).

The chick limb develops on the basis both of cell lineage and of intercellular interactions. The cells which form the myofibrils of the muscles are derived clonally from the somites, and the cells which form all the other tissues are derived from the lateral plate mesoderm (Chevallier, Kieny and Mauger *J. Embryol. exp. Morph.* **41**, 245; 1977). But the spatial pattern of both muscle and non-muscle elements seems to be controlled during outgrowth of the bud by additional mechanisms which act independently along each of the three morphogenetic axes (proximodistal, from the shoulder to the fingertips; anteroposterior, from thumb to little finger, and dorsoventral, from the upper to the lower surface of the limb).

Recent work on the formation of pattern in the proximodistal axis has been dominated by the progress zone model of Summerbell, Lewis and Wolpert (*Nature* **244**, 492; 1973). According to this model, the cells within 200–300  $\mu\text{m}$  of the tip of the limb bud constitute a labile region within which

some metabolic variable called the positional value rises continuously. Because the whole bud is growing, the rate of cell division being particularly high near the tip, cohorts of cells are being displaced continually from the progress zone, and when they leave the zone their positional value becomes frozen. The subsequent development of each cell then depends both on its ancestry (muscle or non-muscle) and on its positional value. This is the 'weak' form of the model. There is also a 'strong' form which asserts that the positional value of a cell in the progress zone is equal to the number of cell divisions through which it has passed since the commencement of outgrowth, and that the  $n$ th skeletal element in the final limb consists of a cohort of cells which all underwent  $n$  divisions in the progress zone.

The original experimental basis of this model involved grafting the tips of wing buds onto stumps of different ages. This led to a controversy between the laboratories of Wolpert in London (*Nature op. cit.*) and Kieny in Grenoble (Kieny and Patou Roux' *Arch.* **179**, 327; 1976; *J. Embryol. exp. Morph.* **41**, 137; 1977). The former group claimed that in these combinations each part developed according to its normal fate map (mosaic development) while the latter group claimed that deficiencies or excesses resulted in a reprogramming of the tissue and a re-establishment of the normal pattern (regulative development). When war between Britain and France seemed inevitable, the deadlock was broken by the dispatch of Summerbell to repeat the experiments in Grenoble. In the resulting concord (*J. Embryol. exp. Morph.* **41**, 137; 1977), both groups accepted that regulation occurs up to stage 22 but not later. At this stage of development the progress zone occupies a large proportion of the bud and so the existence of some interactions is not incompatible with the model.

This removed the strongest objection to the progress zone model, but all embryologists know that a demonstration of mosaic behaviour is not sufficient to prove a positive point because all theories of pattern formation involve a mosaic phase in between the time at which rudiments are first established and the time at which they become visibly differentiated. So a quite different type of experiment has been carried out in Wolpert's laboratory and the results are now reported (*J. Embryol. exp. Morph.* **50**, 175; 1979). Chick wing buds were subjected to a dose of X-irradiation sufficient to kill a certain proportion of their cells and the irradiated buds were allowed to develop after transplantation to healthy embryos. In the resulting limbs the proximal parts were reduced or absent



while the distal parts were well formed. The more intense the dose of radiation the greater the extent of the affected region. This is explained by arguing that the radiation kills a certain fraction of cells throughout the bud. Rudiments which are laid down shortly after irradiation are reduced in size for two reasons: first because a proportion of their cells has been killed, and second, because displacement from the progress zone is slowed down by the reduction in the proportion of dividing cells more distally. Because only the surviving cells proliferate, the progress zone eventually becomes repopulated by healthy cells and so the rudiments which leave the zone last, which are the most distal parts, develop to their normal size.

Does this work provide support for the progress zone model? It does indicate that the rudiments of the limb are laid down in proximodistal sequence, and that at a given stage the proximal part of the bud is a mosaic while the distal part is still labile, so to this extent it is compatible with the model. But it does not seem to me to have any bearing on the mechanism of generation of positional values in the rudiments. In particular it does not connect positional values with the cell cycle, for the sequence of divisions undergone by the surviving cells is presumed to be normal. So I think that it would be wrong to conclude that any evidence has been produced in favour of the strong form of the progress zone model.

Let us now turn our attention through 90° and consider the anteroposterior axis of the bud. The mechanism of pattern formation in this axis seems to be somewhat different from that in the long axis. There is a zone of tissue at the posterior edge of both the wing and the leg bud which is called the zone of polarising activity (ZPA). It is so called because when it is grafted to the anterior edge, it influences the surrounding tissues and causes them to form a second set of limb structures which are arranged in a relation of mirror symmetry to the original set (Saunders *Ann. N.Y. Acad. Sci.* **193**, 29; 1972). It has been suggested that the ZPA is the source of a morphogen that can diffuse into the other parts of the bud and is there destroyed. In the steady state situation the morphogen would be present as a gradient of concentration running from posterior to anterior and its function would be to evoke different pathways of cell differentiation at different levels (Tickle, Summerbell and Wolpert *Nature* **254**, 199; 1975). Some progress has been reported towards the chemical

isolation of the putative morphogen by McCabe and Parker (*Dev. Biol.* **45**, 349; 1975; *ibid* **54**, 297; 1976). Because this is probably the best analysed example of a morphogenetic gradient in the whole of embryology it was with some dismay that workers in the field listened to the talk given by Saunders at a meeting of the British Society of Developmental Biology in September 1976. For Saunders, who had discovered the ZPA in the first place, claimed at the meeting that it had no function at all in normal development. One argument in favour of this position was that buds whose ZPA had been removed at an early stage could go on to produce limb structures which, although often incomplete, were arranged in the normal manner (Fallon and Crosby *J. exp. Zool.* **193**, 449; 1975). The other was that tissue other than the ZPA, for example flank mesoderm or mesonephros, could show the same activity, indicating that the property is not very specific.

To counter these objections to the morphogen model, Summerbell has now reported an experiment designed to demonstrate the activity of the ZPA *in situ* without the need to graft it to an unnatural position (*J. Embryol. exp. Morph.* **50**, 217; 1979). This is done by inserting a small piece of tantalum foil into the bud at right angles to the anteroposterior axis so as to interrupt the transmission of any signal. In the limbs which develop after this operation structures are usually present only on one side of the barrier and when the barrier is placed near the centre of the bud the sequence of structures is truncated in the anteroposterior direction. The explanation for this is that the concentration of morphogen rises on the side of the barrier nearer the ZPA and falls on the side away from it. So a structure which is formed in response to a particular morphogen level will be deleted if its threshold lies in the concentration gap or will be shifted towards the barrier if it is still represented in the interrupted gradient. Summerbell produces computer simulations to demonstrate the reasonableness of this argument, and also describes a control experiment which shows that the gaps in the pattern are not simply the result of damage caused by insertion of the barrier. In many ways the logic and the results of this experiment are similar to those of constriction experiments on insect eggs (reviewed by Sander *Adv. Insect Physiol.* **12**, 125; 1976) which are also believed to indicate the existence of a morphogen gradient with a high point at the posterior end.

It would certainly be unfortunate if the ZPA did not have a role to play in normal development because it has now been detected in the limb buds of

mammals, reptiles and amphibia as well as birds and has found its way into a number of textbooks. But plausible though it is, it is difficult to see how the morphogen gradient model can be proved conclusively by macroscopic experiments alone, and whether the chick embryo limb bud will be capable of supporting the investigations down to the microscopic level of cellular physiology and biochemistry still remains to be seen. □



## A hundred years ago

ON the night of Sunday, May 25, loud bellowings were heard by the dwellers on the northern slopes of Etna. Towards the morning of the 26th these increased, and about midday a dense cloud of smoke was seen to issue from the side of the mountain below the great crater, apparently half way between Randazzo and Linguaglossa. This cloud increased, and on the 27th the mountain was rendered invisible, and an effect like that of an eclipse resulted. A rain of fine black ash, "like powdered emery," fell for miles around, and was so thick that Capo di Schiso could not be seen from Taormina, distance of two miles. This black rain continued all day accompanied by thundering noises from the mountain. No exact information could be procured concerning the position of the centre of disturbance, because no one could approach the new craters. During the night of the 27th the ashes continued to fall, and "huge fires could be seen looming through the black clouds"—no doubt the reflection of the molten lava on the smoke above it. It was reported in Piedemonte, a village on the north-east flanks of Etna, that three craters about a mile apart had opened at the points of a triangle, about six miles above Passo Pisciaro, a posting station nearly midway between Randazzo and Linguaglossa. Lava was said to be flowing in a valley to the north of the Val del Bove. On the 28th a great stream of lava was seen from Taormina to be descending the mountain in the direction of Randazzo, "while from the new craters great balls of fire were thrown high in the air, and burst into showers of fire like gigantic rockets, accompanied by thundering explosions." On May 29 the lava was still flowing, but the shower of ash was diminished. The facts, as above stated, were witnessed by an Englishman living in Taormina, 800 feet above the sea, at the north-eastern termination of the flanks of Etna, about fifteen geographical miles from the new craters.

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# review article

## Weak interactions

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*Recent progress in weak interaction physics is reviewed. The status of the field and its prospects for the future are summarised.*

THE last decade has seen a revolution in the theory of weak interactions. Previously, a phenomenological prescription allowed the successful correlation of a large amount of data concerning nuclear  $\beta$ -decay and the decays of long lived elementary particles (muon, pion and the so-called strange particles) as well as neutrino-induced reactions and muon capture by nuclei. All these processes could be attributed to a simple interaction, the self-coupling of a single 'charged current'. However, the theory remained phenomenological in the sense that processes involving multiple weak transitions, or the interplay of weak and electromagnetic interactions, could not be calculated. In contrast, purely electromagnetic processes have long been understood; the theory permits the calculation of effects to any order in the electromagnetic coupling once the value of electric charge has been specified.

The new developments in weak interactions have resulted in the elaboration of a theory with a calculational power<sup>1</sup> equal to that of quantum electrodynamics. However, while measurements of electromagnetic processes have confirmed the theory to as many as six orders in the coupling constant, it is extremely difficult to test weak interaction theory beyond the lowest order. This is because the coupling is indeed weak, and higher order effects are generally beyond the limits of experimental precision. There are some measurable effects involving K-mesons which are forbidden at lowest order in the weak coupling and therefore measure directly the strength of higher order transitions. However, the interpretation of these phenomena is clouded by the effects of strong interactions.

Although they lack a laboratory for direct tests of the calculational power of the theory, most particle physicists are, nevertheless, convinced that they are on the right track. This is because the construction of the theory required the introduction of new interactions in the lowest order and of new elementary particles, neither of which had been observed. The spectacular confirmation of these predictions form the basis for the present confidence that there is at last a true theory of weak interactions. This article will deal with these new phenomena in so far as they are relevant to neutrino physics.

### Neutral currents

The weak transitions observed before 1973 could all be attributed to a mechanism involving the transmission of one unit of electric charge. This concept is best visualised in terms of pairs of elementary fermions such as the electronic leptons ( $e^-$ ,  $\nu_e$ ), the

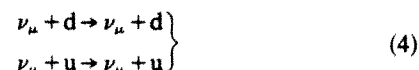
muonic leptons ( $\mu^-$ ,  $\nu_\mu$ ), the lightest quarks (u, d), and the corresponding pairs of antifermions. The members of each pair differ by one unit of electric charge, and 'charged current' processes are those in which charge is exchanged between pairs as, for example, in neutrino induced inverse muon decay:



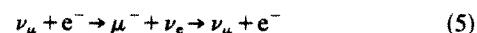
or in neutrino induced reactions involving hadrons, which are ascribed to a charge exchange reaction on quarks, the presumed constituents of hadrons:



The neutral current counterparts of reactions (1) and (2) would be, for example:



Reactions (3) and (4) were long believed to be absent at lowest order in the weak coupling. However, the occurrence of reaction (1) and its inverse implies that reaction (3) should also occur via a doubly weak transition



although at a much lower rate, since the rate for reaction (1) is already very small. The difficulty in constructing a real theory for weak interactions was that if one tried to calculate the rate for reaction (5) it turned out to be infinite.

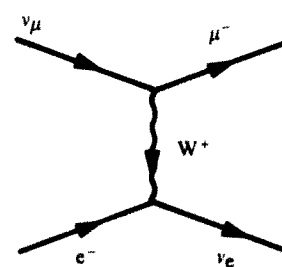


Fig. 1 Inverse muon decay as mediated by a weakly coupled massive vector boson.

The problem is that one must sum over all intermediate ( $\mu^- \nu_e$ ) states in reaction (5), including those that violate energy conservation up to an amount allowed by the Heisenberg uncertainty relation  $\Delta E < \hbar/\tau$  where  $\tau$  is the interaction time. As far as we know from experimental results, the weak interaction is 'point-like'. That is, it has zero range and is instantaneous. That means that intermediate states of infinite energy contribute to reaction (5) with no damping, and the sum does not converge. This problem is avoided by introducing a finite space-time extension for the weak interaction, which is consistent with the data as long as the range introduced is shorter than distances which can be probed by present experiments. In quantum field theory the range of an interaction is governed by the mass of the mediator field: in quantum electrodynamics the mediator is the massless photon and hence the interaction range is infinite. For weak interactions the mediator must be sufficiently massive so that the effects of a non-zero range ( $\tau \sim r/c \sim \hbar/mc^2$ ) are beyond the reach of present experiments. For the conventional charged current interactions, Fig. 1, the presumed mediator is a charged vector boson  $W^\pm$ , and its mass must be greater than about  $20 \text{ GeV } c^{-2}$ .

The introduction of the charged bosons  $W^\pm$  suffices to damp high energy contributions ( $\Delta E \geq m_W c^2$ ) to the sum over intermediate states in reaction (5). At the same time we acquire new weak processes like the production of a pair of charged bosons via the exchange of a lepton, Fig. 2:

$$\nu_\mu + \bar{\nu}_\mu \rightarrow W^+ + W^- \quad (6)$$

While the  $W$ s must be so massive that their production would be difficult at present energies, we can consider higher order processes like

$$\nu_\mu + \bar{\nu}_\mu \rightarrow W^+ + W^- \rightarrow \nu_\mu + \bar{\nu}_\mu \quad (7)$$

again involving a sum over all energies of the intermediate  $W$ - $W$  system. Now a new difficulty arises. Experimental studies of weak processes have demonstrated that the intermediate boson must carry one unit of intrinsic angular momentum (spin), like the photon. However, unlike the photon the  $W$  is massive and therefore has three degrees of freedom instead of two. The amplitude for production of longitudinally polarised  $W$ s grows with energy like  $E/m_W c^2$ , so that the sum over intermediate states is not damped in spite of the space-time extension of the interaction in Fig. 2.

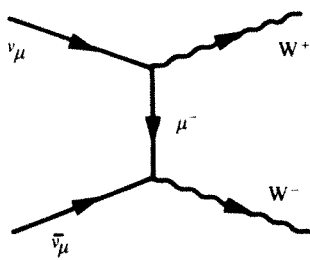


Fig. 2 Charged current muon exchange mechanism for  $\nu_\mu \bar{\nu}_\mu \rightarrow W^+ W^-$ .

We are left with a situation where a manageable theory of weak interactions cannot be constructed without introducing new processes which can interfere destructively with that of Fig. 2, so as to eliminate the high energy contributions to the sum in reaction (7). The simplest possibility<sup>2-4</sup>—and the one apparently chosen by nature—involves the introduction of a massive, electrically neutral spin-1 intermediate boson, the  $Z^0$ , which couples both to the elementary fermions (quarks and leptons) and also to the charged intermediate bosons. Then the mechanism of Fig. 3 can interfere destructively with that of Fig. 2, provided the coupling constants are suitably adjusted. Similar analyses of scattering processes involving charged fermions show that they

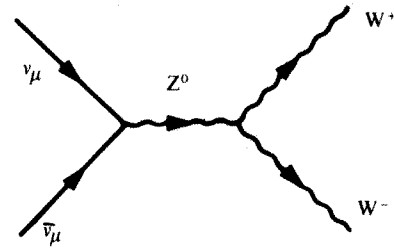


Fig. 3 Neutral current  $Z^0$  exchange diagram for  $\nu_\mu \bar{\nu}_\mu \rightarrow W^+ W^-$  which can cancel Fig. 2 to damp the reaction at high energy.

must also couple to the  $Z^0$ , but with a coupling strength that depends on their electric charge. This is because, for example, the process

$$e^+ + e^- \rightarrow W^+ + W^-$$

can also occur via photon exchange, Fig. 4a, and it is the sum of the three mechanisms of Fig. 4 which must cancel at high energies.

The above arguments mean that the couplings of the  $Z^0$  are tightly constrained in terms of the known charged current and electromagnetic couplings. The  $Z^0$  couples to a fermionic current which is an admixture of a part derivable from the charged current, involving only left-spinning fermions and right-spinning antifermions (that is a  $V-A$  current), and a part which is identical in structure to the (spin independent) electromagnetic current. The relative strengths of these two components depends on the relative strengths of the  $W^\pm$  coupling constant  $g$  and the electromagnetic charge  $e$ . Since low energy charged current reactions measure only the effective Fermi coupling constant,

$$\frac{G_F}{\sqrt{2}} = \frac{g^2}{8m_W^2} \approx 10^{-5}/m_p^2 \quad (8)$$

(where  $m_p$  is the mass of the proton), the theory is characterised by an unknown parameter which is commonly phrased in terms of a 'weak angle'  $\theta_w$ :

$$\sin \theta_w = e/g \quad (9)$$

The current coupled to the massive neutral boson  $Z^0$  is then decomposed according to

$$J^Z = J^{(V-A)} + \sin^2 \theta_w J^{\text{em}}, \quad (10)$$

where  $J^{\text{em}}$  is the electromagnetic current and  $J^{(V-A)}$  is the neutral counterpart of the charged currents. At low energies, exchange of a massive  $Z^0$  will appear as the point like self coupling of the current (10), generating transitions like (3) and (4).

Such processes were first observed<sup>5</sup> in 1973, only after their theoretical importance was realised, and provided an incentive for intensive searches. They had not been observed earlier because of the inherent experimental difficulties. Charged current transitions were extensively studied in decays long before neutrino experiments became feasible. A decay process which can occur via neutral currents, for example a nuclear transition with  $(e^+e^-)$  rather than  $(e\nu)$  emission, is completely masked by the much faster electromagnetic transitions. (This is not the case for strangeness-changing decays which we will discuss later; in fact it was the failure to observe neutral current transitions in strange particle decays which led most particle physicists to assume that only charge changing weak processes were present in nature.) After about 10 years of accelerator neutrino physics, neutral current reactions remained hidden. Neutrino scattering from electrons as in reactions (1) and (3) has a tiny cross-section compared with neutrino scattering from hadrons, and without the final state muon which signs the neutrino hadron scattering process (2), the analogous neutral current process (4) remained buried under neutron-induced background reactions.



Since their discovery<sup>5</sup> by the Gargamelle bubble chamber collaboration at CERN, neutrino-induced neutral current reactions have been studied in great detail<sup>6</sup>. The neutrino-hadron reactions which we have written in terms of the simple quark transitions (4) is translated in the laboratory to the very complex neutrino-nucleon interactions

$$\nu_\mu + N \rightarrow \nu_\mu + X \quad (11)$$

where  $N$  is a neutron or a proton and  $X$  is any allowed hadronic final state, from a single nucleon to a multi-particle system. Studies of a variety of final states have been carried out for both neutrino and anti-neutrino induced reactions. Experiments have also been performed to study the rarer  $\nu_\mu e^-$  and  $\bar{\nu}_\mu e^-$  elastic scattering processes, requiring the observation of an isolated electron track in a particle detector. In addition, the  $Z^0$  exchange contribution to electron-nucleon interactions has been observed<sup>7</sup> via its interference with the dominant photon exchange process. The cross-section for

$$e + N \rightarrow e + X \quad (12)$$

is found to depend on the polarisation of the incident electron, a parity violating effect which signals the contribution of a weak transition.

The simple fact that neutral current processes exist in nature does not provide evidence that cancellation mechanisms of the type described above are really at work. What is striking about the data is that they apparently confirm the precise spin- and charge-dependence of the neutral current coupling as prescribed by the requirement of such cancellations. In other words, each experiment can be analysed assuming the form of equation (10) for the neutral current, and that experiment will provide a determination of the weak angle  $\theta_w$ . The different processes studied yield values for  $\theta_w$  which are compatible with one another within the experimental uncertainties yielding an average value<sup>6</sup>  $\sin^2 \theta_w = 0.23 \pm 0.02$ .

The parameter  $\theta_w$  governs only the relative strengths of the components of the neutral weak current. There is a second measurable parameter which governs the overall coupling strength and is the neutral current analogue of the Fermi coupling constant, Equation (8), for charged current reactions. Constraints were obtained for the neutral current couplings by requiring cancellation of reactions at high energies where masses play no part. Those arguments only constrained the neutral current coupling relative to a particular combination of the  $W$  and photon couplings. The effective Fermi coupling for low energy neutral current reactions is therefore dependent on the  $Z^0$  mass; explicitly one finds

$$\frac{G_F^0}{\sqrt{2}} = \frac{g^2}{8 \cos^2 \theta_w m_Z^2} \quad (13)$$

However, the neutral boson mass  $m_Z$  is not completely arbitrary. The construction of a calculable theory is more delicate than our simple discussion suggests. To ensure that the required relationships among coupling constants remain stable against all higher order corrections, scalar bosons must also be introduced, and the masses of the vector bosons depend on the scalar (Higgs particle) content of the theory. The simplest possibility<sup>3,4</sup> for constructing a fully calculable theory containing three heavy vector bosons requires the introduction of a single scalar particle. In this case the vector boson masses satisfy the relation:

$$m_W^2 = m_Z^2 \cos^2 \theta_w \quad (14)$$

resulting in identical Fermi coupling strengths for charged and neutral current transitions:

$$G_F^0 = G_F \quad (15)$$

Measurements of the neutral current coupling strength satisfy this relation to within the experimental accuracy of about 5%.

To summarise: (1) neutrino experiments have revealed the existence of an interaction long thought to be absent: weak transitions with no exchange of electric charge. (2) The proper-

ties of this interaction are compatible with the requirement that higher order weak effects be calculable. (3) The experimental evidence strongly suggests that weak interactions have the simplest possible form which can satisfy the criterion of calculability.

## Charm and dileptons

We have already paired the elementary fermions according to

$$(e^-, \nu_e), (\mu^-, \nu_\mu), (u, d)$$

This is not quite correct. The up quark ( $u$ ) is actually paired with a superposition of the down quark ( $d$ ) and strange quark ( $s$ ),

$$(u, d_c); d_c \equiv \cos \theta_c d + \sin \theta_c s \quad (16)$$

where  $\theta_c$  is a phenomenological parameter known as the Cabibbo angle<sup>8</sup>. Equation (16) means that the sum of the squared amplitudes for, say, the semi-hadronic transitions

$$d \rightarrow u + e^- \bar{\nu}_e \quad (17)$$

$$s \rightarrow u + e^- \bar{\nu}_e \quad (18)$$

equals the squared amplitude for the purely leptonic transition

$$\mu^- \rightarrow \nu_\mu + e^- \bar{\nu}_e \quad (19)$$

which is the  $\mu$ -decay process. Reaction (17) is responsible for neutron and pion  $\beta$ -decay, while reaction (18) is responsible for the  $\beta$ -decay of strange particles. Comparison of the decay rates determines the Cabibbo angle:  $\sin \theta_c \approx 0.2$ . One of the most striking features of strange particle decays is the very strong suppression of neutral current transitions. For example, the neutral current process

$$K_L \rightarrow \mu^+ + \mu^- \quad (20)$$

is found to be suppressed relative to the charged current process

$$K^- \rightarrow \mu^- + \bar{\nu}_\mu \quad (21)$$

by a factor  $10^{-8}$  in the decay rate.

On the other hand, if we pursue the analysis described in the previous section, we see that the higher order quark-scattering process

$$\bar{d}s \rightarrow W^+ W^- \rightarrow \bar{d}s \text{ (or } \bar{s}d) \quad (22)$$

will have an infinite amplitude unless the mechanism of Fig. 5a for  $\bar{d}s \rightarrow W^+ W^-$  is cancelled by another mechanism, which one might assume to be the  $Z^0$  exchange mechanism of Fig. 5b. However, this would imply an effective neutral current Fermi coupling strength for strangeness changing processes as strong as that for charged current ones, and in particular the decay of reaction (20) would be as rapid as the decay (21). As this is in gross disagreement with the data, the only way to render the process (22) finite is to introduce yet another process. The simplest possibility is to introduce a new quark<sup>9</sup>  $c$ , with couplings to the  $d$  and  $s$  quarks such that the  $c$ -exchange diagram of Fig. 5c cancels the  $u$ -exchange diagram of Fig. 5a. This is achieved if the new quark, called a charmed quark, couples to the  $d, s$  combination which is orthogonal to the one coupled to  $u$ . Thus we introduce a new fermion doublet

$$(c, s_c); s_c \equiv \cos \theta_c s - \sin \theta_c d \quad (23)$$

To ensure that the delicate cancellations arranged for weak interactions are not destroyed by strong interaction effects, the charmed quarks must be subject to the same strong forces as the ordinary  $u, d$  and  $s$  quarks. This means that they should also form bound states. The fact that no charmed hadrons had been observed could be attributed to a higher mass for the charmed quarks and hence for their bound states. However, for this picture to be consistent with kaon data, the charmed quark mass could not be made arbitrarily high. The cancellation of the

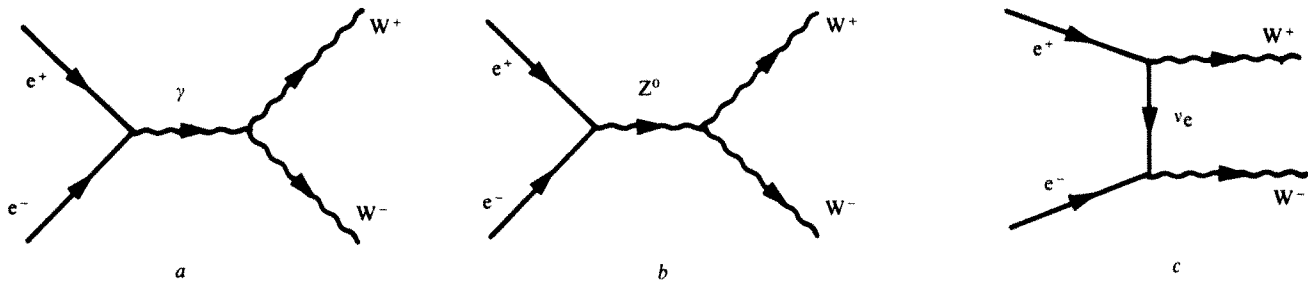


Fig. 4 Mechanisms for  $e^+e^- \rightarrow W^+W^-$  which must interfere destructively at high energies in a calculable theory.

processes in Fig. 5a and c is only exact if the up and charmed quark masses are equal. Therefore, if  $m_c \gg m_u$ , the sum over intermediate  $W^+W^-$  states in reaction (22) will be damped only for energies  $E > m_c c^2$ . This is directly relevant to  $K$  decay; in the absence of a direct  $Z^0 \bar{s}d$  coupling, the decay (20) can still occur via higher order mechanisms such as

$$\bar{s}d \rightarrow W^+W^- \rightarrow \mu^+\mu^-$$

which will have an effective Fermi coupling determined by the charmed quark mass:

$$G_F^{\text{eff}} \sim \cos^2 \theta_c \sin^2 \theta_c G_F^2 m_c^2 \quad (24)$$

The tiny rate observed for the decay (20) is consistent<sup>10,11</sup> only with a charmed quark mass no greater than one or two  $\text{GeV } c^{-2}$ .

The startling discovery of the  $J/\psi$  particle with a mass of  $3.1 \text{ GeV } c^{-2}$  at Brookhaven<sup>12</sup> and at SLAC<sup>13</sup> in 1974 was immediately interpreted by several particle physicists as a charm-anticharm quark bound state. However, it took about two years before this identification was firmly established, and the study of the  $J/\psi$  family of  $\bar{c}c$  states led to considerable progress in the understanding of strong interaction dynamics. We describe here, however, how the weak couplings of charmed particles were studied, particularly in neutrino experiments. Once again these couplings are completely determined<sup>9,14</sup> by the requirement that the theory be calculable (cancellation between Fig. 5a and c), and they are characterised by two features: (1) the (c, s) coupling dominates the (c, d) coupling by a factor  $\cot \theta_c \approx 5$  in amplitude; (2) the charged current coupling is of the usual V-A type.

How are charmed particles produced in neutrino experiments? The nucleon is visualised as a system of bound quarks: three valence quarks which determine its quantum numbers (uud for the proton, ddu for the neutron) and quark-antiquark pairs which are referred to as the quark 'sea'. A neutrino incident on a nucleon can create a charmed quark via reactions like

$$\nu_\mu + d \rightarrow \mu^- + c \quad (25)$$

$$\nu_\mu + s \rightarrow \mu^- + c \quad (26)$$

where in reaction (25) the d can be a valence or a sea quark, but the strange quark in reaction (26) must come from the sea. The

weak interaction breaks up the nucleon, and the charmed quark picks up, say, a  $\bar{u}$  or  $\bar{d}$  antiquark from the hadronic debris to form a charmed D meson. The D meson will then decay weakly, mainly via the strangeness changing processes

$$c \rightarrow s + \{\mu^+ \nu_\mu \text{ or } e^+ \nu_e \text{ or } u \bar{d}\} \quad (27)$$

One therefore expects to find K mesons in the final state when a charmed meson has been produced. This prediction has been verified both in  $e^+e^-$  pair creation of D mesons<sup>15,16</sup> (via the electromagnetic process  $e^+e^- \rightarrow \gamma \rightarrow \bar{c}c$ ) and in neutrino production<sup>17,18</sup> of a single charmed particle. When the D decays into a system of only charged hadrons, it can be identified directly by the reconstruction of its mass ( $1.86 \text{ GeV } c^{-2}$ ) from the momenta of its decay products. If the D decays semi-leptonically some of its energy is taken by the unobserved neutrino. In this case the event is signed by an extra lepton in the final state. Such events were found<sup>6</sup> as expected to be characterised by an excess of kaons.

Even without identification of the hadrons in the final state, it is possible to verify the expectations (1) and (2) simply by studying dimuon events:

$$(\nu_\mu \text{ or } \bar{\nu}_\mu) + N \rightarrow \mu^- + \mu^+ + X \quad (28)$$

where X represents the final state hadronic system for which only the total energy is measured. The important elementary transitions contributing to dimuon events are reactions (25) and (26), followed by the decay

$$c \rightarrow s + \mu^+ + \nu_\mu \quad (29)$$

and antineutrino production of anticharm from the  $\bar{s}$  sea:

$$\bar{\nu}_\mu + \bar{s} \rightarrow \mu^+ + \bar{c} \rightarrow \bar{s} + \mu^- + \bar{\nu}_\mu$$

Antineutrinos can also produce  $\bar{c}$  from  $\bar{d}$  antiquarks in the nucleon's sea. However, the sea contribution is small, and scattering from s and  $\bar{s}$  is important only because it is favoured by the factor  $\cot \theta_c$  in amplitude. There can also be a charm-anticharm component of the quark sea, but ( $c\bar{c}$ ) pairs can be created only for a time  $\tau \sim \hbar/2m_c c^2$  which is small compared

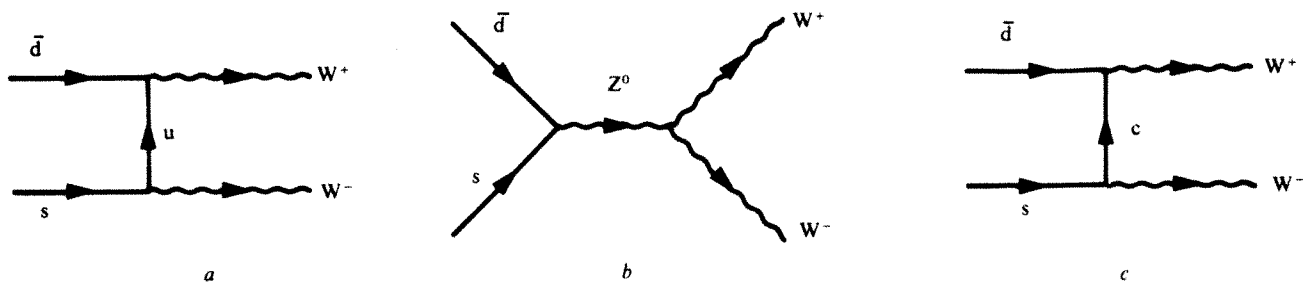


Fig. 5 Mechanisms which might interfere so as to damp the strangeness changing reaction  $d + s \rightarrow W^+ + W^-$ ; a, Conventional charged current mechanism; b, neutral current coupling which is disallowed by K-decay data; c, charmed quark exchange.

with the interaction time unless the neutrino energy is well above the threshold for charm production.

Assuming that scattering from the charmed quark sea is unimportant, we expect the following characteristics for dimuon events.

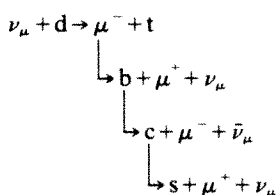
(1) Because of the larger amplitude for scattering from an  $s$  or  $\bar{s}$  quark, the sea contribution is enhanced relative to that for ordinary single muon events. On average the sea quarks carry a smaller fraction of the nucleon momentum than ordinary quarks, so dimuon events should be characterised by a lower effective centre of mass energy for the scattered  $\mu^-c$  or  $\mu^+\bar{c}$  system. This effect should be most pronounced in  $\bar{\nu}_\mu$  induced events where sea quarks make the only contribution.

(2) The final state muon angular distribution depends on the relative spin alignments of the initial interacting fermions. The distribution is isotropic in the neutrino-quark centre of mass system if their spins are both aligned (right spinning) of both anti-aligned (left spinning) with their direction of motion. This is the case for the three processes (25), (26) and (30) if the coupling to charmed quarks are of the usual  $V-A$  type as predicted.

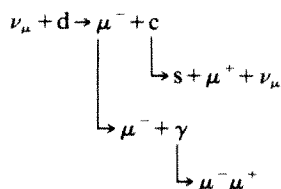
A few dimuon events, suggestive of charm production, were observed<sup>19</sup> even before the discovery of the  $J/\psi$ . By now several high statistics studies have been performed and they support<sup>6</sup> both the predictions (1) and (2) which verify, respectively, conditions (1) and (2) given previously.

## Beyond charm: multileptons

The addition of a charmed quark along with  $u, d$ , and  $s$  established a lepton-quark symmetry for weak interactions: for each lepton pair there is a corresponding quark pair. This symmetry is actually necessary<sup>20</sup> if the theory is to be fully calculable; otherwise infinite amplitudes are found to occur for processes like  $e^+e^- \rightarrow W^+W^-$  when higher order contributions are included. Therefore, the discovery<sup>21,22</sup> of the  $\tau$ -lepton and its associated neutrino led to the anticipation that still another quark pair ( $t, b$ ) should exist. This anticipation received partial confirmation in the discovery of the  $Y$  with a mass of  $9.4 \text{ GeV } c^{-2}$ , generally interpreted as a  $\bar{b}b$  bound state—leaving the  $t$  still to be discovered, presumably with a higher mass. Just as  $c$  couples preferentially to  $s$  in charged current transitions,  $t$  will couple preferentially to  $b$ , and it is not unlikely that  $b$  will decay more often to  $c$  than to  $u$ . Then cascade decays can generate spectacular multi-lepton events like



Unfortunately, the charged couplings of  $b$  and  $t$  quarks to the valence  $u$  and  $d$  quarks are constrained by other data to be very small, so that production cross-sections will not be large. Quadrimuon events have already been observed, but these data can be understood in terms of charm production accompanied by the Bremsstrahlung of a virtual photon, for example



It is hoped that beneath the background from this now 'conventional' source of multimuoons, a few events signalling still newer physics may be dug out.

## Prospects for weak interactions

Assuming that no experimental surprises will upset what now seems to be the steady confirmation of a true theory of the weak interactions, what is the future of the field?

(1) A crucial test of the theory which is still out of reach of present experimentation is the establishment of the actual existence of the postulated vector bosons,  $W^\pm$  and  $Z^0$ . If the theory is correct their masses are already known from the measured values of the weak angles  $\theta_w$ , the Fermi constant  $G_F$ , and the electric charge  $e$  through equations (8), (9) and (14). This gives

$$m_W = (77.8 \pm 3.4) \text{ GeV } c^{-2}, \quad m_Z = (88.7 \pm 2.7) \text{ GeV } c^{-2}$$

New facilities are being constructed at CERN and at Fermi Lab with the primary goal of producing and detecting these particles. A further important step would be their pair production by very high energy electron-positron colliding beams which would allow a probe of the WWZ coupling.

(2) The theory also requires the existence of one or more scalar particles, but little can be predicted about their masses or even their number, although recent speculations concerning the unification of strong, weak and electromagnetic interactions in terms of a single force (see below) place the mass of a scalar particle at about  $10 \text{ GeV } c^{-2}$ . Finding any scalar particle with the appropriate properties<sup>23</sup> would be an important confirmation of present theoretical ideas.

(3) Some theorists believe that detailed studies of hadrons containing the heavy  $b$  and  $t$  quarks may shed new light on the origin of CP violation, which so far has manifested itself only in the isolated neutral kaon system.

(4) The present theory contains an arbitrary parameter, the weak angle  $\theta_w$ . This parameter is indeterminate except within the context of an enlarged theory containing new couplings and new vector bosons. Much theoretical effort is being devoted to the construction of a theory which not only determines  $\theta_w$ , but describes the strong, weak and electromagnetic forces in terms of a unique coupling constant. There has been considerable progress in this direction, but the experimental predictions which could test such a theory are up to now extremely limited, and even higher energies do not promise much more. The most exciting possibility at present is that the proton may be unstable and have a lifetime which is accessible to experimental measurement.

(5) The outstanding problem is an almost total lack of understanding of the fermion spectrum. How many fermion pairs are there? Why are their masses so disparate? Are neutrino masses absolutely zero? Why do quarks form weakly interacting pairs according to particular superpositions of quark states? Some of these questions have been partially considered by the attempts to unify forces discussed above. Some of their answers may also be related to the scalar particle content of the theory. But the general theoretical stalemate with respect to the elementary fermions suggests most persistently that further surprises may be in store.

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# articles

## Mid-Mesozoic closure of Permo-Triassic Tethys and its implications

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*Data from the northern Tethyan domain indicate that Permo-Triassic Palaeo-Tethys closed between the late Triassic and the mid-Jurassic. This closure was caused by the collision with Laurasia of a Cimmerian continent rifted away from northern Gondwanaland during the Triassic. The Neo-Tethys may have opened partly as a back-arc basin over the Palaeo-Tethyan subduction zone and rotation of the Cimmerian continent may have been partially responsible for mid-Mesozoic block-faulting in extra-Alpine central Europe.*

HSÜ<sup>1,2</sup> suggested that the Tethyan Ocean, contemporaneous with the latest Palaeozoic-early Triassic Pangaea<sup>3-5</sup> was obliterated mainly during the Jurassic along a suture zone that must lie north of the late Mesozoic-Cenozoic sutures of the Tethyan orogenic system. This is an attractive hypothesis because all the late Mesozoic-Cenozoic sutures within the Tethyan Orogen represent oceans (hereafter Neo-Tethys) that opened during the Triassic and later<sup>6</sup> (Fig. 1). They cannot therefore mark the site of the latest Palaeozoic-early Triassic Tethys (hereafter Palaeo-Tethys; Tethys 1 of Dewey *et al.*<sup>5</sup>). I review evidence here from the Alpine/Himalayan orogenic system for the existence and mid-Mesozoic closure of Palaeo-Tethys and discuss some tectonic implications of this event.

### Regional review

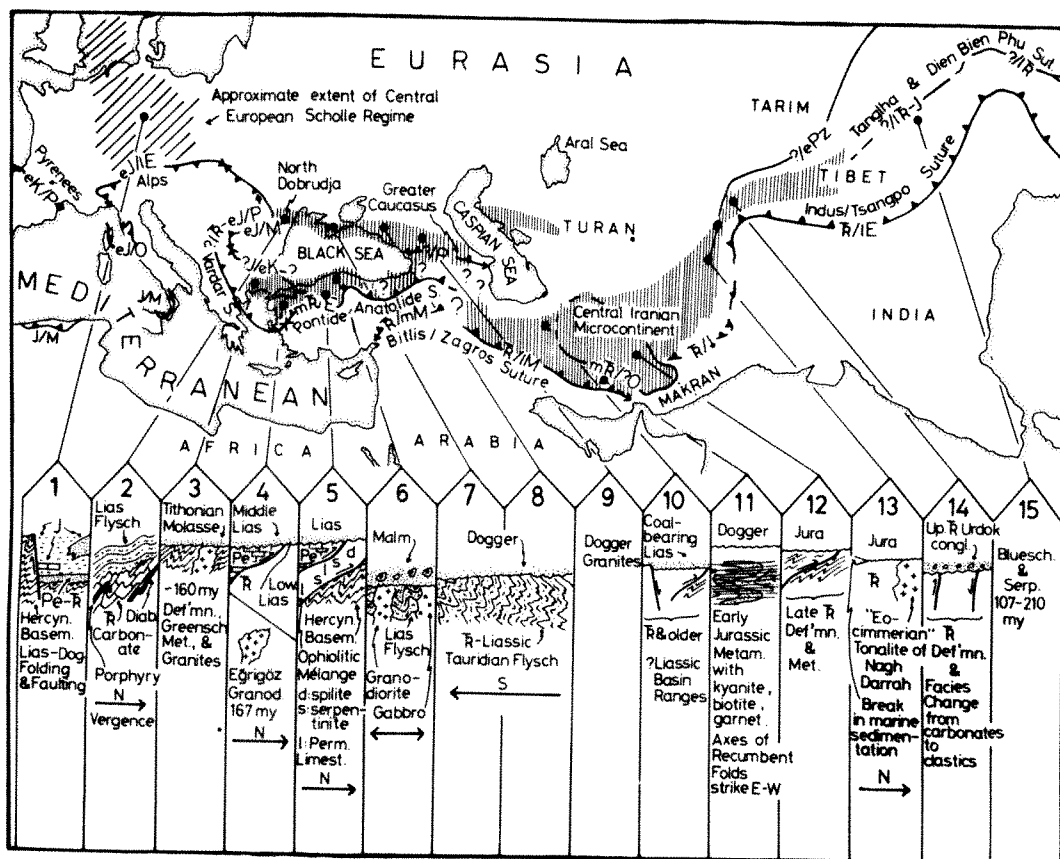
The existence of Palaeo-Tethys is implied by the observation that when the continents around the Atlantic and Indian Oceans are restored to their positions before the break-up of Permo-Triassic Pangaea<sup>3-8</sup>, a large, westward narrowing triangular area appears between Laurasia and Gondwanaland. Figure 1 shows that all of the Alpine-age sutures south and west of Dobrudja-Crimea-Greater Caucasus represent oceans that opened during the Triassic or later (Pyrenees: early Cretaceous<sup>9</sup>; Alps/Apenines: early Jurassic<sup>10</sup>; Carpathians/Vardar Ocean: late Triassic and/or early Jurassic<sup>5</sup>; Pontide/Anatolide Ocean: mid-Triassic<sup>11,12</sup>; Bitlis/Zagros Ocean: Triassic<sup>13</sup>; North Makran/Afghanistan and Indus/Tsangpo Oceans: Triassic<sup>14</sup>). These Neo-Tethyan oceans mostly ruptured northern Gondwanaland. As far west as the Balkans they cut terrains characterised by Pan African/Baikalian or older basement, and from the Balkans westwards they cut Hercynian basement (ref. 15, Fig. 6). Evidence, both from Atlantic and Indian Ocean magnetic anomaly

lineations<sup>5,8</sup> and from palaeogeographic analyses of the peri-Tethyan domain<sup>6,15</sup> shows that the Permo-Triassic Palaeo-Tethys must have lain north of the Alpine sutures, separating late Palaeozoic Laurasia from late Palaeozoic Gondwanaland.

No pre-Triassic ophiolite complexes have been confirmed within the Alpine System<sup>1-5</sup> and therefore it has been difficult to locate the site of the closure of Palaeo-Tethys. Because of the sparsity of ophiolites I use here a variety of criteria such as compressional deformation, Barrovian metamorphism, calc-alkaline magmatism, rapid emergence and flysch/molasse facies sedimentation collectively to identify a zone within which I suggest the Palaeo-Tethyan suture is located. One of the chief reasons why the suture zone has so far gone undetected is that it lay very close to the northern margin of Neo-Tethys and the very intense deformation associated with the closure of that complex ocean has largely overprinted the signature of the earlier collision. Figure 1 shows areas thought to have been affected by the deformation associated with the closure of Palaeo-Tethys. This area is widest where terminal Alpine collision has not yet occurred (north of Makran), an observation that supports the view that the Alpine collisions have very strongly overprinted the earlier structures.

The westernmost places where the effects of early to mid-Mesozoic convergent plate margin activity can be seen are the peri-Rhodopean Belt in the northern Aegean (Fig. 1, loc. 3; refs 16, 17) and in North Dobrudja (Fig. 1, loc. 2; ref. 18 and the bibliography therein). The precise geometry of the mid-Mesozoic deformation in the peri-Rhodopean Belt is not well-understood, but Kockel *et al.*<sup>15</sup> have reported intense folding, greenschist metamorphism and granite intrusion sometime during the Dogger-Malm transition. The deformed assemblage is overlain by Tithonian molasse<sup>17</sup> indicating that here deformation had long ceased when the Eo-Hellenic tectonism began in the sub-Pelagonian area in post-Tithonian times<sup>17</sup>. Whereas the peri-Rhodopean deformation may be related to the closure of Palaeo-Tethys, the Eo-Hellenic ophiolite obduction is clearly pre-collision Neo-Tethyan. In North Dobrudja the geometry is much clearer and involves a north-vergent, imbricated and folded series that includes Alpine-type Triassic and possibly early Liassic carbonates and flysch-facies deposits (Nalbantian Flysch). Porphyry and diabase dykes were intruded during the Trias. Middle Lias is unconformable on this deformed and intruded assemblage. The entire deformed package, making up the Tulcea Zone, is overridden from the south along a major thrust<sup>18</sup> by the Macin Zone that was deformed largely during the Palaeozoic. Because the Baikalian (late Proterozoic) Moesian Platform with its gently deformed Phanerozoic cover is now

**Fig. 1** The approximate area (vertically ruled) affected by mid-Mesozoic orogeny and thought to have coincided roughly with the present extent of the rocks representing the Cimmerian continent(s), the collision of which with Laurasia is believed to have caused the mid-Mesozoic orogeny. Localities for which stratigraphic, structural and petrologic data are given in the columns (1–15). The bold lines are sutures (dashed where inferred). Polarity information, where available, is indicated by black teeth on the overriding continent. Ages on the sutures are ages of ocean opening and closing for the segments limited by arrowheads and abbreviated as follows: Pz, Palaeozoic; T, Triassic; J, Jurassic; K, Cretaceous; P, Palaeocene; E, Eocene; O, Oligocene; M, Miocene; ↓, active subduction; e, early; m, medial; l, late. In the columns Pe denotes Permian. Horizontal zig-zag lines are unconformities.



located between North Dobrudja and the peri-Rhodopean Belt, relationships between these provinces are not clear.

Eastwards, along the strike from Dobrudja, the Triassic to Liassic Tauridian Flysch in the Crimean Mountains (Fig. 1, loc. 7; refs 19, 20) exhibits a strong, south-vergent fold and imbricated thrust structure<sup>21</sup> unconformably overlain by Dogger sandstones. The same stratigraphy and structural picture is exhibited in the basement of the northern slope of the West Kubanian foredeep (Fig. 1, loc. 8; ref. 22), where a thick Middle–Upper Triassic flysch with volcanics has been folded during the Trias–Lias transition. Although similar lithologies of the same age have been encountered in deep drill-holes farther east (eastern shore of the Caspian Sea, Fig. 1, ref. 19), the structural picture there is not known. A strip is thus roughly defined north of the Black Sea characterised by deformed Triassic–Liassic flysch with volcanoclastic components and by diabase and porphyry intrusions.

Parallel with this strip, the southern shores of the Black Sea exhibit stronger, predominantly north-vergent mid-Mesozoic orogenic activity (at this time the Black Sea had not opened and its southern shores may have been much closer to the strip defined above, depending on the position of the Moesian Platform at the time). Around Balya in western Turkey (Fig. 1, loc. 4, ref. 23) Radelli mapped Permian limestones lying with thrust contact on Triassic and Lower Liassic rocks. The thrusts are truncated by an unconformity over which Middle Lias was deposited. Just east of Balya, north of Simav, the Eğrigöz granodiorite gives a Rb–Sr whole-rock age of  $167 \pm 14$  Myr (ref. 24). In the Karagöl region, north of Ankara (Fig. 1, loc. 5; ref. 25), the same Permian limestones are overthrust onto an ophiolitic mélange containing spilites, serpentinites and blocks of the Permian limestones; this package, in turn, is seen in thrust contact on Hercynian basement and all are unconformably overlain by Lias. Other than the northeastern Pontide ophiolites of northeastern Turkey (see below), the suspected pre-Mesozoic ophiolite slivers of Mashad<sup>1</sup>, northern Iran, and the serpen-

tinities along the Tanglha suture in Tibet (Fig. 1, loc. 15), this ophiolitic assemblage is the only occurrence I know that may represent Palaeo-Tethyan ocean floor material. Farther north-east, around the towns of Küre, Inebolu and Devrekâni (Fig. 1, loc. 6; ref. 26), dioritic, granodioritic and gabbroic plutons have been mapped to intrude Liassic flysch. Pebbles of these intrusives are encountered in the basal conglomerate of transgressive Malm sequences. Dogger intrusions of 'granitic' composition are also known from the Greater Caucasus (Fig. 1, loc. 9; ref. 22). Blumenthal<sup>27</sup> has pointed out that the northernmost ophiolite outcrops in the northeastern Pontides in Turkey are of pre-Liassic age.

In central Iran there is abundant evidence for late Triassic–early Jurassic convergent plate–margin activity and Stöcklin has emphasised the importance of the 'cimmerian' movements in this sector of what he has designated the Central Domain, where (Fig. 1, loc. 10; refs 28, 29) late Triassic block-faulting is generally associated with thrusting and intense local folding. This deformation has often been cited as evidence for the rifting of central Iran from Arabia<sup>5,29</sup>. However, the following observations indicate plate convergence: (1) the existence of strongly tectonised regions displaying Barrovian metamorphism (kyanite–biotite–garnet assemblages), accompanying deformation characterised by recumbent similar folding with a strong axial plane schistosity striking east–west in the southeastern extremity of the Sanandaj–Sirjan Zone (Fig. 1, loc. 11; refs 30, 31); (2) strong folding, thrusting and metamorphism of the basement of the Lut Block (central Iranian microcontinent of Takin<sup>32</sup>) (Fig. 1, loc. 12; ref. 28). All of these events took place between the late Triassic and the early Jurassic<sup>28,30,31</sup>. The coexistence of strong compressional deformation and the inferred block-faulting in central Iran during the late Triassic times may be explained in two different ways. First, the area of central Iran may have felt simultaneously the effects of the opening of the Bitlis/Zagros Ocean (a part of Neo-Tethys) and the closing of Palaeo-Tethys. A modern example of a similar association is

the Aegean region<sup>33,34</sup>. Conversely, a basin-and-range regime may have existed in central Iran associated with complications of the convergent plate-margin regime related to the closing of Palaeo-Tethys. Similar complications are observed in the Neogene-Quaternary tectonics of the western US<sup>35</sup>. Ultramafic rocks occurring in ?Carboniferous slates near Mashad, in northern Iran, were interpreted by Hsü<sup>1</sup> as possible representatives of the floor of Palaeo-Tethys.

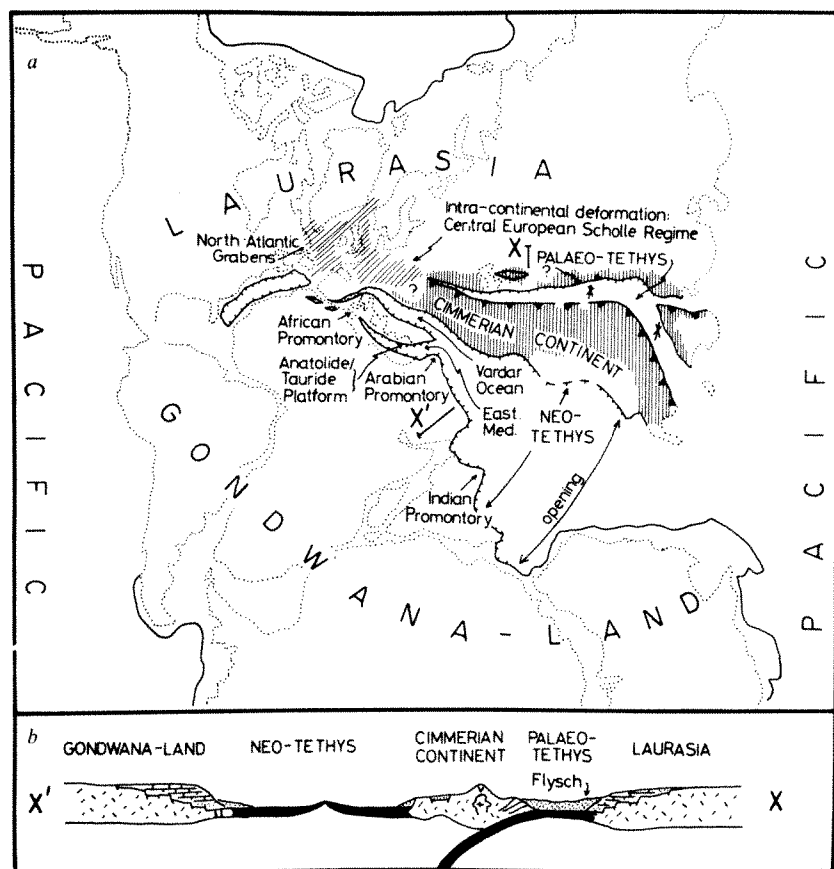
Although the data are sparse and imprecise, in Afghanistan and Pakistan the Triassic-Jurassic transition is characterised by tectonism<sup>36</sup>, a break in marine sedimentation<sup>37</sup> and intrusion of calc-alkaline igneous material such as Salang and Doab granites of western Hindu Kush Mountains (isotopic age:  $210 \pm 10$  Myr; ref. 38). In western Hindu Kush, tectonism is characterised by a strong, north-vergent nappe tectonics of intra-Jurassic age<sup>38</sup> (Fig. 1, loc. 13). Farther east, in the Pamirs and the Karakorum (Fig. 1, loc. 14; refs 28, 39, 40) the late Triassic-early Jurassic orogenic activity is better documented: in the Pamirs the deformation was accompanied by the intrusion of calc-alkaline plutons and by a sharp facies change from shelf carbonates to a coarse clastic facies. In the Karakorum this latter facies is represented by the 'orogenic' Urdok Conglomerate which is the only coarse clastic sediment recognised in the Palaeozoic-early Mesozoic section<sup>40</sup>.

Eastwards, the mid-Mesozoic zone of deformation enters the Tibetan Plateau, the geology of which is little known. Chang and Zeng<sup>41</sup> indicate that there is a Mesozoic arc suture along the Tanglha Mountains (Fig. 1, loc. 15) with serpentinites and blueschists; isotopic ages along the Tanglha Mountains range from 210 to 107 Myr (ref. 41). Alternatively, Sengör and Kidd<sup>42</sup> interpret the entire pre-Jurassic basement of Tibet as a giant accretionary mélange prism, first hit by a continent during the Himalayan collision. The Tanglha suture seems to continue along strike into the late Triassic Dien Bien Phu suture in southeastern Asia (Fig. 1; ref. 43). If the Tanglha suture represents a mid-Mesozoic continental collision (or arc-continent collision), we may continue the zone of mid-Mesozoic

orogeny into Tibet as a suture zone. Conversely, if the hypothesis of Sengör and Kidd<sup>42</sup> is correct, then the Palaeo-Tethyan suture must have joined, from both sides, the large accretionary prism of Tibet, much as the present accretionary prism of Makran<sup>44</sup> links the Zagros and Indus-Tsangpo sutures. Palaeobiogeographic data (summarised in ref. 45) indicate that the welding of southern and northern Tibet occurred sometime between Permian and Jurassic.

## Discussion and implications

The evidence reviewed above, when coupled with the marine magnetic and palaeogeographic arguments for the existence of Palaeo-Tethys, may be interpreted in terms of a late Triassic to medial Jurassic closure of this ocean. This closure occurred between Eurasia and the continental fragment(s) rifted from the northern side of Gondwanaland. Figure 2a shows the simplest possible interpretation of this continental fragment which I call here the Cimmerian continent after the Cimmerian Mountains that comprise the Macin Mountains, the Serpent Island and the Crimean Mountains. Clearly the Cimmerian continent may have had a very different geometry and have been composed of more than one fragment. The lack of precise simultaneity or of a regular age progression along the zone of mid-Mesozoic orogeny support the concept of a segmented Cimmerian continental archipelago and the apparent interruption in the zone of mid-Mesozoic deformation in northwestern Iran, the circum-central Iranian microcontinent suture (Fig. 1), and the evidence that Tibet may be characterised during this interval as a large accretionary prism indicate where such possible discontinuities may have been located. Neo-Tethys has also similarly closed by independent fragments of Gondwanaland. The pre-Alpine internal structure and possible width of the Cimmerian continent(s) are particularly difficult to assess because of the very strong Alpine overprint. In all areas where the mid-Mesozoic closure of Palaeo-Tethys caused deformation (Fig. 1) there has also been both intense Alpine orogenic deformation



**Fig. 2** a, Latest Triassic-early Jurassic reconstruction of Pangaea and Tethys (base map from ref. 54) showing the simplest possible interpretation of the internal geometry of the Tethyan realm. All the elements shown, particularly the poorly-defined Cimmerian continent, could have had different and probably more complex geometries. Vertically ruled area corresponds to that in Fig. 1, that is it represents the regions affected by mid-Mesozoic orogeny. Here the width of the Cimmerian continent is purely hypothetical and may be as much as three times too large. Obliquely ruled areas are regions of complex intracontinental extension inferred to have been partially related to the closure of Palaeo-Tethys. Lines with black triangles are subduction zones with the triangles on the upper plate. Lines with short hachures represent passive continental margins (no fit is intended between the southern and northern shores of Neo-Tethys due to lack of data). Dotted lines are the present continental shore-lines put in for reference. XX' indicates the location of the cross-section shown in b. b, A highly schematised cross-section showing the major elements considered in the text. Black denotes oceanic and short, irregular lines denote continental crust, +, Calc-alkaline intrusive; v, calc-alkaline volcanic rock.



and exceedingly complex post-collisional foreland deformation<sup>33,34,42,46,47</sup>. These later events and the associated extensive volcanics and Neogene sedimentary cover seem to have obliterated or hidden the pre-Alpine characters of the Cimmerian continent(s) beyond any reasonable hope of coherent synthesis.

Because of the very fragmental and imprecise nature of the data, particularly from Iran eastwards, it is difficult to interpret the polarity(s) of Palaeo-Tethyan subduction zone(s). The location of relatively less deformed and largely unmetamorphosed flysch/molasse sequences to the north of the strongly deformed, metamorphosed and intruded, more 'internide-looking' predominantly north-vergent orogenic terrains as far east as Afghanistan may indicate that at least in this sector the Palaeo-Tethyan orogen was probably north-facing with a south-dipping subduction zone. In Tibet, if the regions north of the Tanglha Mountains are composed of late Palaeozoic-early Mesozoic accretionary mélange material, the subduction zone may have been dipping northward; but here data are so sparse that the question is open.

If the above proposed scheme is approximately correct, then it has interesting implications for two problems outside the Palaeo-Tethyan area. One is the complex, intracontinental zone of extension in extra-Alpine central and northwestern Europe (Fig. 1, loc. 1; refs 48-50). This broad zone of extension, characterised by a complicated pattern of block faulting (Schollen), became active during Liassic (?earlier) time and accelerated during the Malm. I suggest that this predominantly intracontinental extensional regime was possibly caused by the closure of wedge-shaped Palaeo-Tethys. If the pole of rotation of the Cimmerian continent(s) was located somewhere between North Dobrudja and Germany, as suggested by the observation that no early to mid-Mesozoic orogenic activity is known in Europe west of North Dobrudja, then the convergent plate boundary system, if not joined near the pole by other plate boundaries, must have become extensional once it crossed the pole. This extension should have increased away from the pole; indeed the early to mid-Mesozoic extension in the North Sea-North Atlantic regions was far more intense than it was in central Europe<sup>50</sup>. The broad distribution of the associated strain may have been because extension was taking place in continental lithosphere and was following Permo-Triassic intracontinental grabens and shear zones<sup>51</sup>. It may, however, prove impossible to distinguish between extension caused by the initial

opening of the Atlantic and that related to the hinging of Palaeo-Tethys.

The second problem is related to the opening of Neo-Tethys. I suggest that if the Cimmerian continent or substantial segments thereof were sufficiently narrow, that is if they were essentially island arcs, the Triassic opening of Neo-Tethys may be interpreted as the opening of a series of back-arc basins or at least initiated by back-arc spreading processes (Fig. 2b). McKenzie and Weiss<sup>52</sup> have pointed out that it seems particularly difficult to initiate constructive plate margins. They suggested that back-arc spreading may be the triggering mechanism for initiating major oceans, as back-arc spreading and seafloor spreading in major oceans seem to be the same process (see appendix in ref. 53). Although their main example, the South Atlantic, was unfortunate, the idea may be applicable, at least to parts of Neo-Tethys.

## Conclusions

Palaeo-Tethys that must have existed during the Permo-Triassic between Laurasia and Gondwanaland closed during the late Triassic-mid-Jurassic possibly along one or more suture zones located within the zone of mid-Mesozoic orogeny shown in Fig. 1. As a result of very strong Alpine overprinting associated with the latest Mesozoic-Cenozoic demise of Neo-Tethys and because of the sparsity of data neither the precise geometry of the suture(s) nor that of the continent(s) that collided with Laurasia to close Palaeo-Tethys can be reconstructed. However, my interpretation permits a satisfactory explanation of a number of phenomena, notably the following: (1) The mid-Mesozoic convergent plate-margin activity in a relatively narrow band stretching from Rhodope and North Dobrudja to southeastern Asia and the observation that the Alpine-Himalayan sutures represent oceans opened not before the Triassic. (2) The mid-Mesozoic extensional regime in extra-Alpine central Europe which may be viewed as a partial consequence of the convergent plate-margin activity in the Palaeo-Tethyan realm. (3) The opening of Neo-Tethys which may be considered, at least in part, to be an example of back-arc spreading associated with the subduction of the Palaeo-Tethyan ocean floor.

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# Sand on the southern Mediterranean Ridge: proximal basement and distal African–Nile provenance

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*Petrographic analysis of Quaternary terrigenous sand layers in eastern Mediterranean cores reveals distinct mineralogical differences between the Egyptian Shelf–Nile Cone region and the southern part of the Mediterranean Ridge. A compositionally and texturally immature suite in Ridge cores, mixed with a Nile-derived assemblage, identifies a fresh non-recycled mineral component derived from proximal igneous and metamorphic surface or near-surface exposures, probably in the south-central Ridge area rather than from distal African sources. The presence of such basement terrains would be consistent with a compressive thrust-belt origin for this part of the Mediterranean Ridge.*

THE Mediterranean Ridge, the broad arcuate swell in the eastern Mediterranean extending from the Ionian Sea to the eastern Levantine Basin, constitutes a distinct topographic and tectono-stratigraphic province between the seismically active southern European and more stable North African margins. The characteristic hummocky ridge-and-swell topography of the Ridge is the surface expression of what appears in seismic profiles as a folded and faulted sediment complex<sup>1,2</sup>. The contact between deformed sedimentary units of the southern part of the Ridge and the more gently stratified, Plio-Quaternary series forming the western Nile Cone tends to be well-defined<sup>3</sup> and delineates the northwestern margin of the Herodotus Basin plain (Fig. 1). A mid-oceanic ridge origin<sup>4</sup> can be ruled out for the Mediterranean Ridge, but there is considerable diversity of opinion as to the structural and stratigraphic configuration<sup>5–7</sup>. The various hypotheses pertaining to the geological development of this feature are reviewed by Finetti<sup>8</sup> and Stride *et al.*<sup>9</sup>.

All the schemes invoke important large-scale tectonic displacement in the late Tertiary to the present, and one would expect that evidence of these geologically recent events should be recorded in sedimentary sequences deposited in this area. This article focuses on the provenance of terrigenous sand layers of Pleistocene age on the southern part of the Ridge at the approximate midpoint between the more obvious potential sources of sediment: Nile Delta, Crete, Cyprus and the Eratosthenes Seamount. This sector has more specifically been termed the Hellenic Outer Ridge<sup>9</sup>. Pleistocene and possibly Upper Pliocene core sections recovered in this part of the Ridge in 1970 at DSDP Site 130 (Fig. 1) have been interpreted as distal Nile Cone deposits which were uplifted <1 Myr ago and subsequently deformed<sup>2</sup>. Arguments in support of this scheme are based partly on regional correlation of sub-bottom reflectors of probable Messinian and post-Miocene age, but more specifically on mineralogical comparisons between DSDP Sites 130 and 131 at the base of the Nile Cone (ref. 2, p 735 and 1033;

ref. 10) and on the pollen content in Pleistocene samples at Site 130<sup>11</sup>. These studies all emphasise the North African, largely Nile, affinity of the southern Ridge deposits. The difference in relative percentages of the dominant heavy minerals (primarily an amphibole–pyroxene–epidote–opaque mineral suite) between Nile Cone and Ridge samples is attributed by Ryan and others<sup>2</sup> to differences in grain size and to changes of source terrain drained by the Nile River during the Quaternary.

This study considers heavy and light minerals from terrigenous sand layers, mostly turbidites, in DSDP Site 130 and 131 cores and in Pillsbury 6508 and 6510 and Vema 10 piston and gravity cores on the outer Egyptian Shelf, Nile Cone and Herodotus Basin plain, and shallower marine palimpsest deposits in Chain 119 grab samples on the inner to middle Egyptian Shelf in front of the Nile Delta (Fig. 1). Observed regional differences indicate a more complicated provenance-dispersal of Pleistocene sediment on the southern part of the Mediterranean Ridge than previously envisioned.

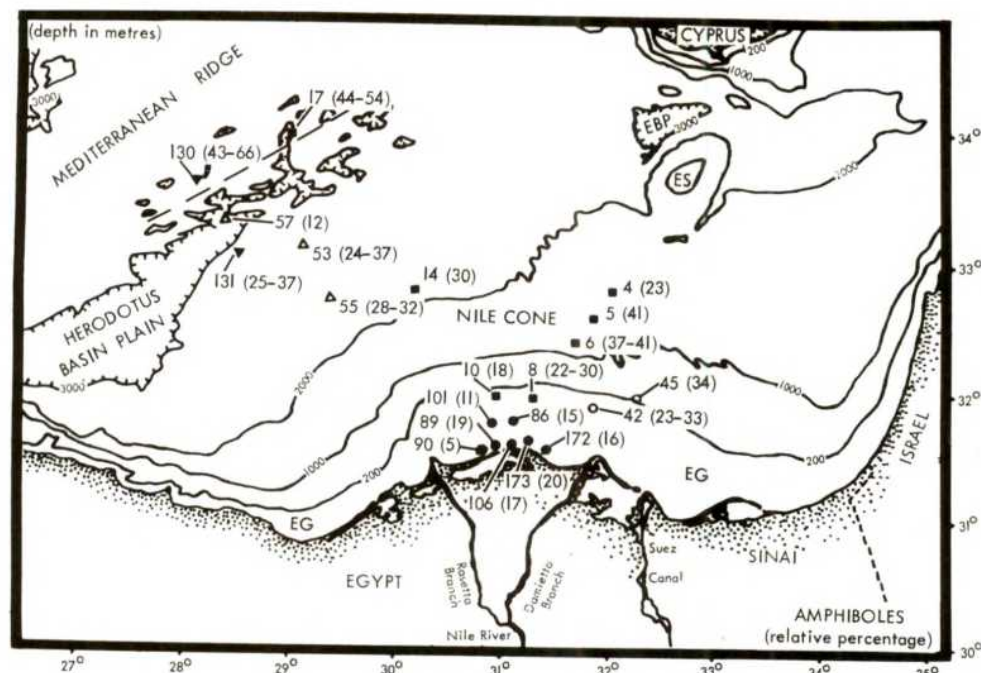
## Petrographic observations

Most Pleistocene sand layers in the Nile Cone and the Mediterranean Ridge core sections are of bioclastic origin<sup>12</sup>, and our investigation considers only terrigenous or mixed terrigenous–bioclastic layers. Of about 225 sand layers sampled in the cores shown in Fig. 1, only 47 examined had a sufficiently heavy mineral content for grain count (Table 1). Data are based on the calculated 62 to 250 µm heavy mineral fraction of each layer to minimise size-sorting effects. Heavy fractions were separated by the standard heavy liquid method and 300 grains were identified. The listed data shows that the study area occupies a province dominated by pyroxenes, amphiboles, epidotes and opaque minerals; these four account for more than 90% of the total heavy minerals in most samples, as was indicated in earlier investigations<sup>2,10,13,14</sup>.

Vertical changes were observed in some cores (Table 1): for example, in DSDP Site 131 from the lower Nile Cone, there is a slight downcore increase in pyroxenes and epidotes and an increase in amphiboles; in Pillsbury core 6510–18 on the outer Egyptian Shelf, there is a slight downcore increase in pyroxenes and amphiboles, and a more random change in epidote content. Regional–spatial changes are more apparent and include a general decrease in the relative percentage of opaque minerals between the outer edge of the shelf and distal Nile Cone stations. Overall, the Nile Cone samples in grabs, short piston and gravity cores and in DSDP Site 131 are genetically related to the same general assemblage as the Nile River valley, Nile Delta and coastal sequences<sup>13–17</sup>.

The most pronounced changes in heavy mineral assemblages occur between the inner and outer Egyptian Shelf and between the base of the Nile Cone and the southern part of the Mediterranean Ridge. These regional distinctions become evident when considering the ranges of relative percentages of minerals in the





**Fig. 1** Chart of the southeastern Mediterranean showing sample station locations on the Egyptian Shelf (EG), Nile Cone and southern part of the Mediterranean Ridge. ES, Eratosthenes Seamount; EBP, Eratosthenes Basin plain. Numbers refer to core and grab sample station and, in parentheses, the relative percentage of amphiboles in the non-opaque heavy mineral assemblage (data from Table 1). Note increased amphibole content in Ridge samples. ●, Chain 119; ○, Pillsbury 6508; ■, Pillsbury 6510; △, Vema 10; ▼, DSDP Leg 13.

three major topographic sectors of the study area (Table 1):

(A) On the Egyptian Shelf (10 stations): clinopyroxenes (8.5–62.8), orthopyroxenes (0.0–7.9), amphiboles (5.0–33.3), epidotes (6.5–37.0), garnets (0.0–46.3), zircon–tantalum–rutile, or ZTR (0.0–6.5), apatite (0.0–1.6), opaques (23.0–70.0).

(B) On the Nile Cone proper (nine stations): clinopyroxenes (12.9–46.5); orthopyroxenes (0.0–3.3); amphiboles (11.9–40.8); epidotes (4.8–30.6); garnets (0.0–2.5); ZTR (0.0–4.7); apatite (0.0–1.6); opaques (11.0–60.0).

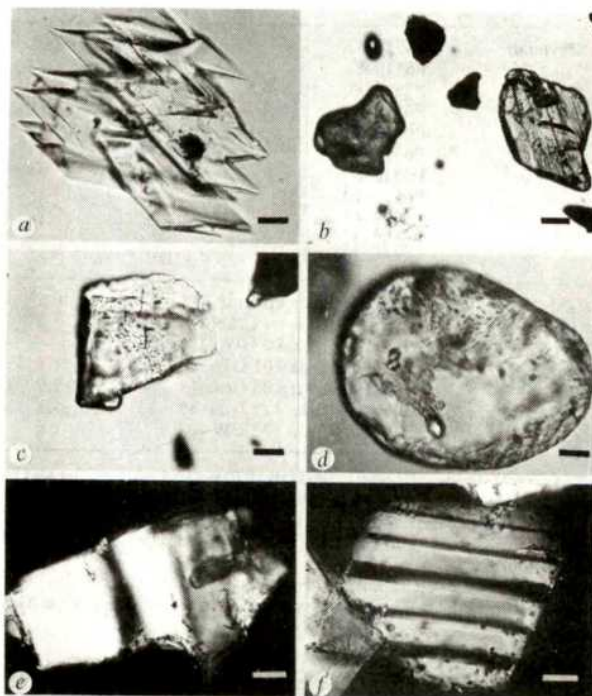
(C) On the southern Mediterranean Ridge (two stations): clinopyroxenes (10.0–17.3); orthopyroxenes (0.4–2.0); amphiboles (43.4–65.8); epidotes (8.5–26.5); garnets (0.4–3.6); ZTR (0.4–3.6); apatite (1.6–2.2); opaques (47.0–68.0).

Nile Cone (B) samples display a narrower range in relative percentage and smaller core-to-core differences (particularly clino- and orthopyroxenes and garnets) than the Egyptian Shelf (A) samples. The southern Ridge (C) samples (DSDP Site 130 and Pillsbury 6510–17) are distinguished from Nile Cone–Herodotus Basin plain (B) samples by enhanced proportions of amphiboles and lower percentages of pyroxenes as noted earlier by Ryan *et al.*<sup>2</sup> and Bartolini *et al.*<sup>10</sup>. Moreover, a careful evaluation of specific heavy mineral types in our Ridge core sample separations reveals other significant differences: a somewhat higher proportion of brown hornblende, serrated or saw-tooth pyroxenes (Fig. 2a), angular apatite (Fig. 2c); the presence of a few glaucophane grains; and a markedly higher proportion of both garnet and opaque minerals in the DSDP Site 130 samples. Examples of the more typical rounded pyroxene and apatite grains in the Nile Cone are shown for contrast in Fig. 2b and d.

The compositional differences between sands in the lower Nile Cone–Herodotus Basin plain sector and those on the southern part of the Ridge also are recorded in the light mineral fractions. There is a relatively higher proportion of fresh to slightly altered and angular feldspar grains on the Ridge (Fig. 2e) than in DSDP Site 131 and other Nile Cone core samples where subangular to rounded altered grains prevail (Fig. 2f). In DSDP Site 131 and other Nile Cone core samples, there is a strong wavy extinction: normal–weakly wavy extinction ratio of about 2:1. Two samples of DSDP Site 130 contain quartz with primarily normal or weakly wavy extinction, and three with strongly wavy extinction; most quartz in Ridge core P6510–17 also displays strong wavy extinction.

More distinct regional differences are recorded by the nature and frequency of quartz inclusions: samples from DSDP Site

130 and P-6510–17 tend to show fewer solid (rutile needles, zircon, apatite) and fluid–gaseous inclusions than in DSDP Site 131 and Nile Cone and outer Shelf samples (Fig. 3a, b) and in comparable Nile Delta sands as earlier detailed by Kholief<sup>18</sup>. Some quartz grains from Ridge core P6510–17 (sands sampled at 149 and 452 cm from top of core) and DSDP Site 130 (sample 4–2) are characterised by angular overgrowths over euhedral and subhedral crystals (Fig. 3c), unlike some quartz from the



**Fig. 2** Photomicrographs showing details of some heavy and light minerals from the southern Mediterranean Ridge (a,c,e) and Nile Cone (b,d,f) core samples. a, Saw-tooth amphibole (Pillsbury 6510–17, 452 cm) scale bar, 10 μm; b, subrounded amphibole (DSDP Site 131–6, 129–131 cm) scale bar, 100 μm; c, angular apatite (DSDP Sites 130–5–3, 125–126 cm) scale bar, 25 μm; d, rounded apatite (DSDP Site 131A–4–2, 117–119 cm) scale bar, 15 μm; e, fresh, angular plagioclase feldspar (Pillsbury 6510–17, 452 cm) scale bar, 15 μm; f, subrounded, worn-edged plagioclase feldspar (Vema 10–57, 690–691 cm) scale bar, 15 μm.



**Table 1** Heavy mineral data from samples collected on the southern Mediterranean Ridge, Nile Cone and Egyptian Shelf

Region	Core no.*	Depth† (cm)	Relative percentage of heavy non-opaque minerals									% Opaques	% Non-opaques
			Orthopyroxenes	Clinopyroxenes	Amphiboles	Epidote	Garnet	ZTR‡	Sphene	Apatite	Others§		
Southern sector of Ridge	13-130-4-2	96-97	0.9	17.3	65.8	8.5	0.9	0.4	—	1.7 <sup>a</sup>	4.5	47.0	53.0
	13-130-5-2	90-91	1.8	16.4 <sup>c</sup>	43.4	26.5	1.3	3.1	0.4	1.8 <sup>a</sup>	5.3	68.0	32.0
	13-130-5-3	125-126	0.8	13.5 <sup>c</sup>	59.8 <sup>b</sup>	10.4	3.6	3.6	—	1.6 <sup>a</sup>	6.7 <sup>d</sup>	51.0	49.0
	P6510-P17	149	2.0	10.0 <sup>c</sup>	53.8 <sup>b</sup>	21.3	0.4	1.6	0.8	—	10.9 <sup>d</sup>	8.0	92.0
	P6510-P17	452	0.4	14.5 <sup>c</sup>	44.1 <sup>b</sup>	21.1	0.9	2.2	—	2.2 <sup>a</sup>	14.6 <sup>d</sup>	28.0	72.0
Western Nile Cone-Herodotus Basin Plain	13-131-1-6	18-20	0.4	46.5	25.2	18.4	2.0	2.0	—	1.6	3.9	41.0	59.0
	13-131-1-6	129-131	0.4	41.3	25.4	24.1	2.5	4.7	—	0.4	1.2	42.0	58.0
	13-131A-1-2	55-57	0.3	45.6	32.9	16.1	0.3	0.3	1.4	0.3	2.8	32.0	68.0
	13-131A-1-2	81-83	0.4	37.2	32.1	25.8	2.0	—	1.2	—	1.3	24.0	76.0
	13-131A-1-2	122-124	0.4	43.1	36.5	15.4	0.4	0.4	—	0.4	3.4	28.0	72.0
	13-131A-1-2	145-147	—	43.8	30.5	15.4	1.3	1.3	0.8	—	6.9	25.0	75.0
	13-131A-4-2	8-10	—	43.9	29.2	18.3	1.4	1.0	0.3	0.3	5.6	34.0	66.0
	13-131A-4-2	117-119	—	45.4	24.9	26.5	1.8	1.1	—	—	0.3	26.0	74.0
	13-131A-4-2	137-139	—	43.7	34.1	18.5	0.3	0.7	—	—	2.7	21.0	79.0
	V10-53	330-331	2.5	30.2	23.5	16.9	0.4	2.1	—	—	24.4	28.0	72.0
	V10-53	372-373	—	12.9	33.2	16.1	0.9	—	—	0.9	36.0	17.0	83.0
	V10-53	384-385	2.5	26.2	26.9	23.3	—	1.1	0.4	0.4	19.2	24.0	76.0
	V10-53	645-646	0.9	17.9	36.8	16.1	0.9	1.3	—	—	26.1	11.0	89.0
	V10-55	240-241	3.3	25.4	28.2	16.7	0.5	2.9	0.5	—	22.5	30.0	70.0
	V10-55	281-282	—	20.3	29.3	30.6	—	0.4	—	0.4	19.0	20.0	80.0
	V10-55	410-411	0.4	24.3 <sup>c</sup>	31.7	28.4	0.8	0.8	—	0.4	13.2	27.0	73.0
	V10-57	690-691	2.7	35.2	11.9	20.1	—	0.9	—	—	29.2	25.0	75.0
	P6510-P14	1128-1129	2.4	26.4	29.5	16.9	0.8	2.4	0.4	0.4	20.8	22.0	78.0
Eastern Nile Cone	P6508-45	10	—	42.8	34.2	22.8	—	—	—	—	0.2	49.0	51.0
	P6510-P4	582	1.4	22.5	23.0	4.8	—	0.5	0.5	—	47.3	14.0	86.0
	P6510-5	130-131	1.2	40.7	40.6	9.8	—	—	—	1.2	6.5	60.0	40.0
	P6510-6	122-123	—	45.7	37.0	14.4	0.9	1.3	—	—	0.7	30.0	70.0
	P6510-6	123-124	1.8	46.5	37.2	8.2	—	—	—	0.4	5.9	42.0	58.0
	P6510-6	142	—	30.2	40.8	20.1	—	—	0.4	—	8.5	37.0	63.0
Outer Egyptian Shelf	P6510-8	1-2	0.4	50.0	24.5	16.5	2.9	2.9	0.8	—	2.0	41.0	59.0
	P6510-8	5-7	—	48.0	25.0	18.4	3.5	3.4	0.4	—	1.3	38.0	62.0
	P6510-8	84-85	0.4	33.3	22.1	37.0	3.2	0.4	—	0.9	2.7	26.0	74.0
	P6510-8	90-91	—	38.8	24.2	31.1	1.6	2.4	—	1.6	0.3	32.0	68.0
	P6510-8	99-100	—	42.1	24.5	23.1	1.1	1.0	0.7	0.3	7.2	34.0	66.0
	P6510-8	109-111	—	37.7	26.9	26.9	1.2	0.4	0.4	0.4	6.1	38.0	62.0
	P6510-8	112-113	—	45.2	30.3	18.1	1.0	0.7	—	—	4.7	23.0	77.0
	P6510-10	90	—	62.8	18.0	6.5	3.7	—	—	0.4	8.6	37.0	63.0
	P6508-42	2	—	45.1	22.9	20.1	3.8	3.8	—	—	4.3	64.0	36.0
	P6508-42	70	—	39.3	33.3	19.6	2.2	4.4	—	—	1.2	70.0	30.0
	P6508-42	100	—	43.1	26.1	26.6	2.7	0.8	0.4	—	0.3	37.0	63.0
	Inter-Mid Egyptian Shelf	CHN 119-II, sta 86 Grab 11		0.5	18.7	14.9	28.8	13.0	6.2	1.0	—	16.9	46.0
CHN 119-II, sta 89 Grab 13			4.2	12.7	19.3	33.5	0.9	0.9	0.5	—	28.0	29.0	71.0
CHN 119-II, sta 90 UW 24			—	8.5	5.0	12.4	46.3	6.5	—	—	21.3	52.0	48.0
CHN 119-II, sta 101 UW 34			1.5	25.1	10.8	18.7	10.8	4.9	—	—	28.2	42.0	58.0
CHN 119-II, sta 106 Grab 15			7.9	26.5	16.7	22.3	—	2.3	—	0.5	23.8	31.0	69.0
CHN 119-II, sta 172 Grab 35			1.8	41.3	15.5	20.0	0.4	0.9	—	—	20.1	36.0	64.0
CHN 119-II, sta 173 UW 44			2.6	39.3	19.6	20.5	1.3	1.7	1.3	—	13.7	31.0	69.0

\* Key to sample stations: 13 refers to DSDP 1970 leg 13 Sites 130, 131 and 131A, with drill core and section numbers as in ref. 2; P, University of Miami cruise Pillsbury 6508 and 6510 piston and gravity cores<sup>20</sup>; V, Lamont-Doherty Geological Observatory cruise Vema 10 piston cores<sup>12</sup>; CHN, Woods Hole Oceanographic Institution cruise Chain 119 grab samples<sup>19</sup>.

† Depth in centimetres from piston and gravity core tops or DSDP core section top.

‡ Zircon + tourmaline + rutile.

§ Includes altered as well as unidentified grains.

<sup>a</sup> Angular apatite; <sup>b</sup> brown hornblende present to common; <sup>c</sup> angular and saw-tooth pyroxenes; and <sup>d</sup> presence of few glaucophane grains.

Nile Cone and Egyptian Shelf which display partial rounded overgrowths on rounded grains (Fig. 3d). Some angular quartz grains of the Ridge show no evidence of wear. Also significant are angular and euhedral quartz and feldspar grains (found only in DSDP Site 130, samples 4-2 and 5-3) that display a type of radiating crack pattern with fissures that extend from the centre towards the edge of the grain; the intensity of fissuring decreases outward (Fig. 3e). This pattern is in marked contrast with quartz, usually rounded, in the Delta and on the Shelf and Nile Cone, which more commonly displays one or two generations of cracks (Fig. 3f).

## Evidence for mixed Nile-proximal provenance

The mineralogical composition of terrigenous sand-sized material in surficial Egyptian Shelf and Quaternary Nile Cone samples, including DSDP Site 131, are genetically related to a Nile apport. The large station-to-station differences in the relative percentage of heavy minerals in the surficial Egyptian Shelf samples primarily reflect size sorting and density concentration (lag) effects due to sea floor processes that have reworked both modern and relict sediments<sup>19</sup>. During the Pleistocene, Nile-



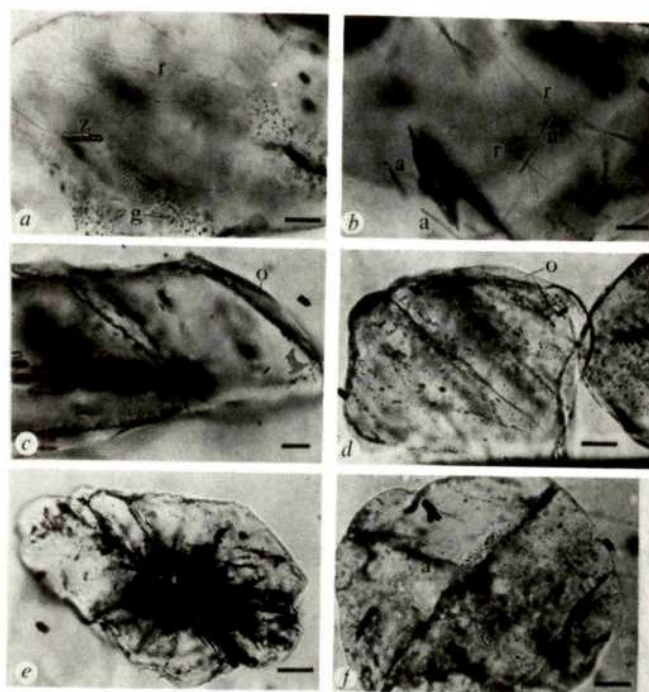
derived sandy fluvial, deltaic and eolian deposits were emplaced downslope by gravity-induced resedimentation processes<sup>2,10,12,20</sup>. The observed fluctuations in relative percentages of heavy minerals between samples within any core and between piston core localities on the different parts of the Nile Cone proper more closely record differences of processes than changes in provenance. This is demonstrated, for example, by the progressive diminution downslope, in the same textural grade, of denser opaque minerals. Both heavy and light mineral suites in Nile Cone Quaternary short piston core and DSDP Site 131 samples are essentially comparable with those described in the main Nile by Shukri<sup>15,16</sup>, and in the sediments of the Delta and related Nile-derived older sections<sup>14,17,21</sup>, which show a contribution (including relatively high percentages of pyroxenes) from the Blue Nile, Atbara and other tributaries draining the Abyssinian volcanic source terrains.

Although composition of the probable lower to middle Pleistocene sands at DSDP Site 130 and Pleistocene sands in Pillsbury core 6510-17 is broadly similar to the Nile-derived sediment on the Nile Cone, our investigation emphasises some significant mineralogical differences on the southern part of the Ridge. We believe that the distinct suite of mineral species in the Ridge samples indicates input from a more proximal source terrain in addition to the Nile-derived sands. The much higher proportion of amphiboles in southern Ridge cores is not, as previously suggested, due only to a finer grain size in this more distal sector; examination of comparable textural grades in Nile Cone DSDP Site 131 and Vema 10-53, 55 and 57 core samples all show substantially lower proportions of amphiboles than in DSDP Site 130 and P6510-17 core samples (Fig. 1). Furthermore, the greater proportion of angular grains of both heavy and light components (apatite, serrated pyroxenes, quartz, feldspar), an increased percentage of unaltered feldspar grains, apatite and brown hornblende, the presence of glaucophane grains, angular quartz overgrowth on euhedral and subhedral quartz grains, a radiating crack pattern within some euhedral quartz and feldspar grains, and a generally lower proportion of solid inclusions in quartz grains identify a mineralogical assemblage distinct from that in the modern to pre-Quaternary main Nile, Nile Delta and Nile coastal deposits.

The angular, serrated and non-altered state of various mineral species in the Ridge samples records minimum weathering-abrasion effects; this is in contrast with the reworked, subangular to rounded and often altered grains on the Egyptian Shelf and Nile Delta that invariably show considerable effect of one or multi-cycle fluvial or eolian (or both) transport. The immature mineral fraction in DSDP Site 130 and P6510-17 sands records the introduction of material from more proximal plutonic igneous and possibly also metamorphic sources rather than from distal African terrains. The compositional mix of reworked Nile-derived material and 'fresh' components would imply the addition of new sediment at some point between the Nile Delta-Egyptian Shelf sector and the Ridge core localities. As most of the sands examined in cores DSDP Site 130 and P6510-17 were emplaced by turbidity currents and associated gravity induced mass-flow processes, we are required to consider some modification of the direct downslope SE to NW dispersal path that has prevailed in this sector during the late Quaternary<sup>12,20</sup>.

## Two-phase dispersal pattern

Where did the fresh sedimentary material enter the transport system? An extensive seismic survey of the Nile Cone by Ross and Uchupi<sup>3</sup> does not reveal basement series at or near the surface between the Nile Delta and the Ridge, and we exclude a southerly position for the introduction of fresh minerals. Transport from a northern Hellenic Arc-Crete source is precluded on the basis of the heavy mineral composition which is significantly different in that sector, a view also adopted by Bartolini *et al.*<sup>10</sup>. Cyprus and the Eratosthenes Seamount, located approximately 300 km NE and E of the sample sites, while not excluded, seem to be unlikely source areas. We can do



**Fig. 3** Photomicrographs showing details of quartz from Nile Cone and Mediterranean Ridge core samples. *a*, Quartz with inclusions of partially altered zircon (*z*), segmented rutile needles (*r*) and orientated minute gas (*g*) bubbles (outer Egyptian Shelf, Pillsbury 6510-10, 90 cm); rutile segments are of variable length and arranged in a regular pattern; scale bar, 55  $\mu$ m. *b*, Quartz with non-orientated inclusions of apatite (*a*) and rutile (*r*) needles (outer Egyptian Shelf, Pillsbury 6510-10, 90 cm) scale bar, 20  $\mu$ m. *c*, Angular secondary overgrowth (*O*) on subhedral quartz, with orientated cracks and green solid inclusions (southern Mediterranean Ridge, Pillsbury 6510-17, 452 cm) scale bar, 10  $\mu$ m. *d*, Rounded secondary overgrowth (*O*) partially surrounding a subrounded quartz grain, which shows a system of orientated cracks with secondary inclusions (outer Egyptian Shelf, Pillsbury 6510-10, 90 cm) scale bar, 55  $\mu$ m. *e*, Subhedral quartz with radiating crack pattern displaying fissures that extend outwards from the centre of the grain (southern Mediterranean Ridge, DSDP Site 130-5-2, 90-91 cm) scale bar, 50  $\mu$ m. *f*, Round quartz grain displaying two generations of cracks lined with secondary inclusions, and an apatite (*a*) solid inclusion (outer Egyptian Shelf, Pillsbury 6510-10, 90 cm) scale bar, 55  $\mu$ m.

little more, at this point, than invoke a two-phase dispersal pattern: transport downslope of recycled African sand on the Nile Cone surface in a direction away from the Nile Delta to an area north of the present Herodotus Basin plain during a first stage of Nile Cone progradation<sup>2</sup>, and then a remobilisation and subsequent short-distance transport of this Nile-derived material to which a distinct igneous and metamorphic fraction has been added in the Ridge area. The topographically complex configuration of the Ridge is of geologically recent origin, and it is possible that some resedimentation occurred during the Pleistocene in the area where transport would not be possible on the present Ridge surface.

Immature mineral assemblages in Pleistocene sands of the short P-6510-17 core, about 90 km NE of the much older Pleistocene DSDP Site 130 locality, strengthens the hypothesis of yet undefined igneous-metamorphic sources in the south-central Ridge area. The possibility of a surface or near-surface exposure of sub-sedimentary basement on the Ridge should not be readily dismissed although no specific evidence for this has been provided by gravity measurements or seismic profiles. Our findings, not inconsistent with compressive thrust-belt<sup>8</sup> and décollement<sup>22</sup> reconstructions, suggest that possibility of some movement of basement, perhaps as part of an allochthonous



slice. The petrographic evidence indicating local input should be considered in developing an interpretive scheme for the Mediterranean Ridge.

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# Characterisation of deletions which affect the expression of fetal globin genes in man

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*Deletions in the DNA of individuals with hereditary persistence of fetal haemoglobin (HPFH) and  $\delta\beta$ -thalassaemia have been mapped as a means of identifying regulatory sequences involved in the switch from fetal to adult globin gene expression. The end points of these deletions have been precisely located with respect to restriction endonuclease cleavage sites within and surrounding the  $\gamma$ -,  $\delta$ - and  $\beta$ -globin genes in normal human DNA and the deletion maps were used to obtain definitive evidence for the physical linkage of the fetal and adult  $\beta$ -like globin genes in the order 5' $\gamma$ - $\delta$ - $\beta$ 3'. Correlation of haematological data and the location of deletions in two cases of HPFH and one case of  $\delta\beta$ -thalassaemia suggest that a region of DNA located near the 5'-end of the  $\delta$ -globin gene may be involved in the suppression in cis of  $\gamma$ -globin gene expression in adults. The interpretation of a second case of  $\delta\beta$ -thalassaemia is complicated by the fact that the deletion removes the  $\gamma$ -gene in addition to the region near the 5'-end of the  $\delta$ -globin gene.*

HUMAN globin genes provide a unique system for studying the nature of mutations which alter the normal programme of differential gene expression during mammalian development. For example, in normal individuals the fetal  $\beta$ -like globin genes, designated  $\gamma$  and  $\delta$ , are turned down at 32–34 weeks of gestation, while the adult  $\beta$ -like genes, designated  $\delta$  and  $\beta$ , are turned up. Certain genetic disorders have been described in which this control 'switch' is altered (see refs 1, 2 for reviews); in that designated hereditary persistence of fetal haemoglobin (HPFH)<sup>1–12</sup>, often neither the  $\delta$ - nor the  $\beta$ -globin chains are synthesised in adult erythroid cells, but one or both of the

$\gamma$ -globin genes continue to be expressed, compensating for the  $\beta$ -globin deficiency. In  $\delta\beta$ -thalassaemia, no  $\delta$ - or  $\beta$ -globin chain synthesis is detected in adults, but in contrast to HPFH, compensation by continued fetal globin synthesis is incomplete. The resulting imbalance of  $\alpha$ - and non- $\alpha$ -globin polypeptide synthesis in homozygous  $\delta\beta$ -thalassaemia leads to anaemia and red cell abnormalities characteristic of thalassaemia syndromes<sup>1</sup>. Differences in the pattern of globin gene expression in individuals with HPFH and  $\delta\beta$ -thalassaemia led to the proposal that sequences necessary for the repression of  $\gamma$ -globin gene expression in the adult might be deleted in HPFH DNA<sup>7</sup>. In fact, when DNAs from individuals homozygous for HPFH or  $\delta\beta$ -thalassaemia were examined by solution hybridisation experiments, extensive deletions of  $\beta$ -globin gene sequences were observed<sup>3–6</sup>. This result was confirmed<sup>9,10</sup> using the blot hybridisation technique of Southern<sup>13</sup> to map single copy genes<sup>14,15</sup>. Mapping deletions in HPFH and  $\delta\beta$ -thalassaemia may provide insights into the mechanism of the switch from fetal to adult globin gene expression.

Advances in physical mapping and gene cloning procedures have made it possible to determine the linkage arrangement of human fetal and adult globin genes and have provided hybridisation probes for examining DNA which lies outside gene sequences. Previous genetic evidence<sup>1,2</sup> and structural analysis of the fusion proteins Lepore<sup>16</sup> and Kenya<sup>17</sup> suggested that the fetal and adult  $\beta$ -like globin genes are linked. Physical linkage of the  $\delta$ - and  $\beta$ -globin genes<sup>18,19</sup> and of the  $\gamma$ - and  $\delta$ -globin genes<sup>20</sup> was demonstrated using blot hybridisation<sup>13</sup> and gene cloning<sup>21</sup> procedures. Physical linkage between  $\delta$ - $\beta$  and  $\gamma$ - $\delta$  gene loci has not been demonstrated.

In this report we use cloned complementary DNA and genomic DNA fragments as probes in blot hybridisation experiments to map the end points of deletions in DNA from individuals homozygous for HPFH or  $\delta\beta$ -thalassaemia. In addition, we unambiguously demonstrate direct physical linkage between the fetal and adult globin genes.



## Deletion mapping

Deletions in two cases each of homozygous hereditary persistence of fetal haemoglobin (HPFH-1 and 2)<sup>3,5,11,12</sup> and of homozygous  $\delta\beta$ -thalassaemia ( $\delta\beta$ -1 and 2)<sup>9,10,22</sup> were mapped relative to restriction endonuclease cleavage sites within and surrounding the  $\gamma$ -,  $\delta$ - and  $\beta$ -globin genes in normal human DNA. The map of these cleavage sites in normal DNA shown in Fig. 1a was derived by analysis of cloned globin genes<sup>19</sup> and by blot hybridisation experiments described here and elsewhere<sup>18,20,24</sup>. To scan the entire  $\beta$ -like gene locus systematically for the location of deletions, blots of each type of mutant DNA were first hybridised individually with *in vitro* labelled  $\beta$ - or  $\gamma$ -globin cDNA plasmids<sup>25</sup> or with a cloned DNA fragment from a region between the adult and fetal globin genes. Precise localisation of the end points of the deletions was then accomplished by additional enzyme digestions as described in detail below.

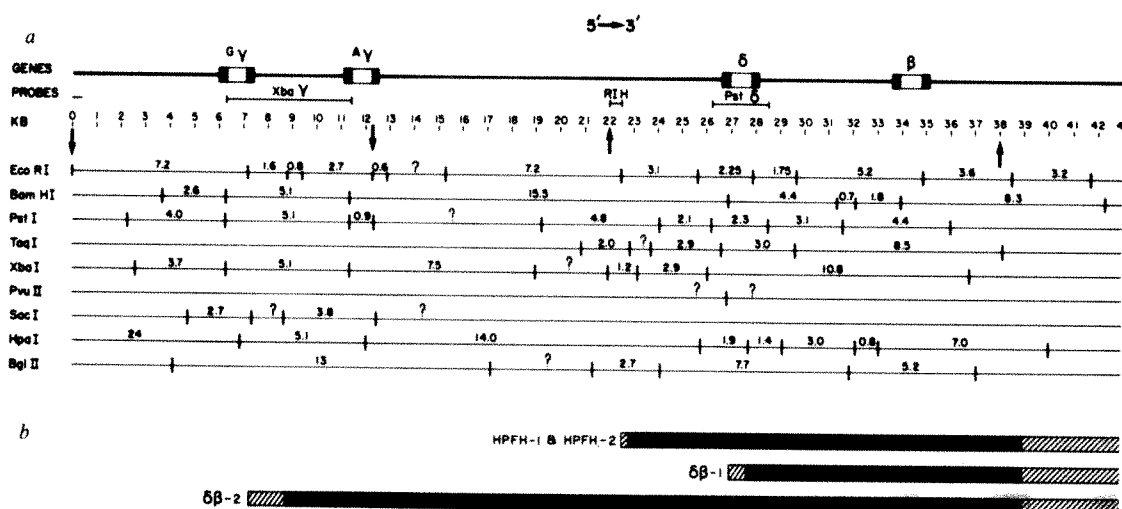
## HPFH-1 DNA

HPFH-1 DNA was obtained from an individual homozygous for a type of HPFH in which both the  $\gamma$  and  $\gamma$  polypeptides are produced in adults<sup>3</sup>. Initially, normal and HPFH-1 DNA were compared by blot hybridisation procedures using the  $\beta$ -globin cDNA plasmid as probe (Fig. 2a). As expected from the restriction map of Fig. 1a, digestion of normal DNA with *EcoRI* produces four fragments (5.2, 3.6, 2.3 and 1.75 kilobases) which hybridise the  $\beta$ -globin cDNA probe (Fig. 2a). Similarly, digestion with *TaqI* produces two hybridising fragments (8.5 and 3.0 kilobases) while digestion with *XbaI* produces a single hybridising fragment (10.8 kilobases). In contrast, no hybridising fragments are observed following digestion of HPFH-1 DNA, indicating that, in agreement with liquid hybridisation<sup>3</sup> and blot hybridisation<sup>10</sup> experiments, HPFH-1 DNA has little or no  $\beta$ - or  $\delta$ -globin gene sequences.

Fragments characteristic of normal DNA were observed when HPFH-1 DNA was digested with *EcoRI*, *BglII* or *SacI* and hybridised to the  $\gamma$ -globin probe (Fig. 2b). These results indicate that the left end point of the deletion in HPFH-1 DNA is located to the left of the  $\delta$ -globin gene as shown in Fig. 1a. We therefore hybridised blots of HPFH-1 DNA with a cloned 0.5-kilobase fragment which lies 4 kilobases to the left of the  $\delta$ -globin gene<sup>19</sup> ('RIH'; see Fig. 1a) to determine whether the RIH sequence is present in HPFH-1 DNA and to map the surrounding restriction enzyme sites. As shown in Fig. 3a, a 7.2-kilobase fragment is observed in both normal and HPFH-1 DNA digested with *EcoRI*, indicating that the RIH sequence is present and that the end point of the HPFH-1 deletion is located to the right of RIH.

To map this end point further, additional enzyme digests were carried out. Digestion of normal DNA with *PstI*, *XbaI* or *TaqI* yields single fragments of 4.8, 1.2 and 2.0 kilobases, respectively, which hybridise to the RIH probe (Fig. 3b). Double digests with *EcoRI* were carried out to map these sites in normal DNA with respect to RIH. Fragments of 3.2 kilobases (*PstI/EcoRI*), 0.5 kilobases (*XbaI/EcoRI*), and 1.6 kilobases (*TaqI/EcoRI*) were observed. These results identify *PstI*, *XbaI* and *TaqI* cleavage sites 3.2, 0.5 and 1.6 kilobases, respectively, to the left of the *EcoRI* site at the right end of RIH. By subtraction from the size of the single digest products, *PstI*, *XbaI* and *TaqI* sites lie 1.6, 0.7 and 0.4 kilobases to the right of this *EcoRI* site (see Fig. 1a).

Similar single and double enzyme digests were carried out with HPFH-1 DNA to determine which of these sites is altered (Fig. 3b). The sizes of the RIH hybridising fragments in the double enzyme digests are identical to those observed with normal DNA, indicating that the *PstI*, *XbaI* and *TaqI* sites to the left of RIH are not altered in HPFH-1 DNA. However, the sizes of all the hybridising fragments produced in single enzyme digests are altered, indicating that sites to the right of RIH are removed by the deletion (Fig. 3b). These data place the end



**Fig. 1** A map of restriction endonuclease cleavage sites within and surrounding the human  $\gamma$ -,  $\delta$ - and  $\beta$ -globin genes. *a*, The arrangement of fetal and adult  $\beta$ -like globin genes within a 43-kilobase segment of human DNA. Physical linkage of the adult  $\delta$ - and  $\beta$ -globin genes<sup>18,19</sup> and the fetal  $\gamma$ - and  $\gamma$ -globin genes<sup>20</sup> has been previously described. The physical linkage of the fetal and adult globin genes is described in the text. The direction of transcription of the four linked genes is left to right ( $5' \rightarrow 3'$ ). The solid boxes represent the locations of mRNA coding regions. The open boxes represent the large non-coding intervening sequence in each gene<sup>18-20,23,24</sup>. This intervening sequence is located between codons 104 and 105 in the  $\delta$ ,  $\beta$  (ref. 19) and  $\gamma$  (ref. 23) genes. Sizes of the  $\gamma$ -,  $\gamma$ -,  $\delta$ - and  $\beta$ -globin intervening sequences are approximately 850, 850, 950 and 900 base pairs, respectively. A smaller intervening sequence identified in the  $\beta$ - and  $\gamma$ -genes and presumably present in the  $\gamma$ - and  $\delta$ -genes is not shown. The brackets below the gene map indicate the positions of the *XbaI*, *RIH* and *PstI* fragments. These fragments are used as hybridisation probes in some experiments and are described in detail in the appropriate figure legends. For each of the restriction endonucleases indicated on the left, the relative locations of cleavage sites are marked by a vertical line. The sizes for the restriction enzyme fragments are given in kilobases. Question marks indicate that the presence of additional cleavage sites has not been determined. The locations of cleavage sites within the region delineated by the upward-pointing arrows were determined from an analysis of the clone HBG1 which contains the linked  $\delta$ - and  $\beta$ -globin genes (ref. 19 and our unpublished results). Some cleavage sites within the region delineated by the downward-pointing arrows were determined by an analysis of the clone HYG1 which contains the  $\gamma$ - and  $\gamma$ -globin genes (unpublished results). Additional cleavage sites within and surrounding the  $\gamma$ -,  $\delta$ - and  $\beta$ -globin genes were mapped by blot hybridisation experiments (refs 18, 20, and this paper). *b*, The regions of the  $\beta$ -like globin gene locus deleted in HPFH and  $\delta\beta$  DNAs are indicated by solid boxes. The precise locations of the end points of the deletions are within the regions specified by the hatched boxes. The locations of the rightward ends of the deletions are not known.

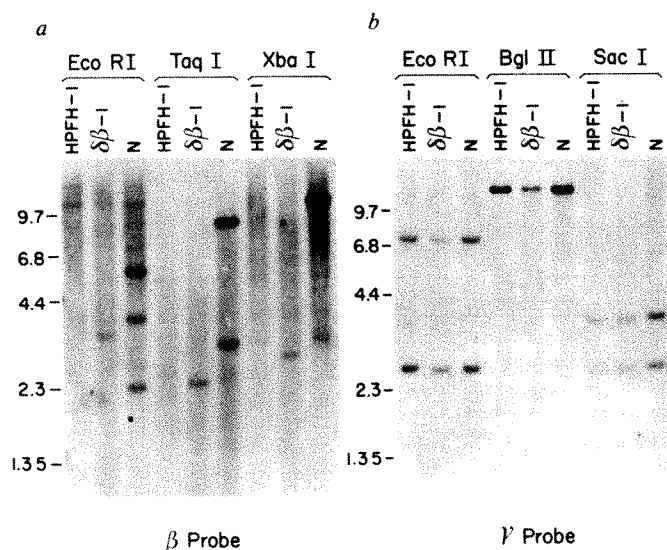
point of the HPFH-1 deletion within the 400-base pair region between the *EcoRI* site bounding the RI H fragment and the *TaqI* site to the right. The deletion therefore extends from approximately 4 kilobases to the left of  $\delta$  to at least 4 kilobases beyond the 3' end of the  $\beta$ -globin gene, which is the rightward limit of our restriction map. Thus, the deletion in HPFH-1 DNA removes at least 16 kilobases of DNA (Fig. 1b).

## HPFH-2 DNA

DNA from a second case of homozygous HPFH was also examined<sup>10-12</sup>. This DNA was obtained from an individual apparently unrelated to the HPFH-1 case. As with HPFH-1, HPFH-2 lacks the  $\delta$ - and  $\beta$ -globin genes and contains no detectable alteration within or closely surrounding the  $\gamma$ -globin genes (data not shown). When HPFH-2 DNA was examined in an experiment identical to that of Fig. 3b, an identical result was obtained (data not shown). Therefore, the deletion in HPFH-2 DNA is indistinguishable from that in HPFH-1 DNA.

## $\delta\beta$ -1 DNA

Hybridisation of  $\delta\beta$ -1 DNA with the  $\gamma$ -globin probe revealed fragments characteristic of normal DNA (Fig. 2b). However, with the  $\beta$ -globin probe (Fig. 2a) a single hybridising fragment was observed in  $\delta\beta$ -1 DNA digested with *EcoRI* (3.0 kilobases) (see also ref. 9), *TaqI* (2.3 kilobases) and *XbaI* (2.5 kilobases). All these sizes differ from those found in normal DNA. This result indicates that a portion of either the  $\beta$ - or  $\delta$ -globin gene is



**Fig. 2** Hybridisation of normal, HPFH-1 and  $\delta\beta$ -1 DNAs with cloned  $\beta$ - and  $\gamma$ -globin cDNA probes. Human DNA was isolated from transformed lymphoid cells<sup>3,9</sup> using the procedure of Blin and Stafford<sup>26</sup>. DNA samples were digested separately with *EcoRI*, *TaqI* or *XbaI*, electrophoresed in a horizontal 0.8% agarose slab gel and transferred to nitrocellulose filter paper<sup>13</sup>. The *HhaI* fragments containing  $\beta$ - or  $\gamma$ -globin cDNA sequences were isolated from the plasmids pJW102 or pJW151 (ref. 25), respectively, labelled with <sup>32</sup>P by nick translation<sup>27</sup> and hybridised to the filter-bound DNA<sup>13,15</sup>. (The recombinant DNA containment level of P3<sup>+</sup> EK2 was used as outlined in the National Institutes of Health Recombinant DNA Research Guidelines.) Following hybridisation the filters were washed sequentially in 1×, 1/2× and 1/4× washing buffer at 68 °C (1× buffer is 50 mM NaCl, 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.1% SDS) and exposed to X-ray film using two DuPont Cronex lighting plus intensifier screens. Bacteriophage  $\lambda$  DNA digested with *HindIII* (ref. 19) and pBR322 DNA digested with *HinfI* (ref. 28) were included in the gel as size standards. The positions of some of the markers are indicated to the left of the autoradiogram (in kilobase pairs). *a*, Digests of  $\delta\beta$ -1, HPFH-1 and normal (N) DNA hybridised with the  $\beta$ -globin probe. The position of the weakly hybridising 1.75-kilobase 3'- $\delta$  *EcoRI* fragment is indicated by the dot. *b*, Digests of the same DNAs hybridised with the  $\gamma$ -globin probe. Two additional *EcoRI* fragments (1.6 and 0.6 kilobases)<sup>20</sup> are not detected by the  $\gamma$ -globin cDNA plasmid used here, which lacks a portion of the 3'-mRNA sequence.

present in  $\delta\beta$ -1 DNA. Hybridisation of *BamHI*-digested  $\delta\beta$ -1 DNA with the RI H probe revealed a fragment identical to that found in normal DNA (data not shown). This result suggests that  $\delta\beta$ -1 DNA might contain 5'- $\delta$ -gene sequences, as the *BamHI* site nearest to the RI H fragment is located within the  $\delta$ -gene. The restriction enzyme *PvuII* was used to demonstrate the presence of 5'- $\delta$ -sequences of  $\delta\beta$ -1 DNA. A *PvuII* site is present in the 5'-end of the  $\delta$ -globin gene and no other *PvuII* sites are found in either the  $\beta$ - or  $\delta$ -globin genes<sup>18</sup>. To detect  $\delta$ -globin gene sequences we used a cloned *PstI* fragment ('*Pst*  $\delta$ '; see Fig. 1a) which contains the  $\delta$ -globin gene and surrounding sequences.

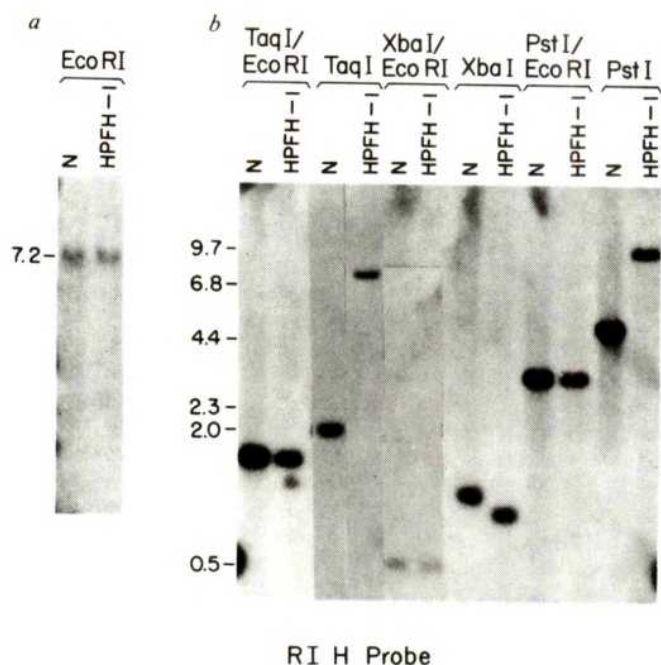
Digestion of  $\delta\beta$ -1 DNA with *PstI* produces a single fragment of 10.5 kilobases which hybridises to the *Pst*  $\delta$  probe. Fragments of 7.3 and 0.9 kilobases are produced in a *PstI*/*PvuII* double digest (Fig. 4a). The presence of a fragment which is cut by *PvuII* to produce two fragments which hybridise the  $\delta$ -globin probe indicates that a portion of the  $\delta$ -globin gene is retained in  $\delta\beta$ -1 DNA. A similar conclusion was obtained from *EcoRI* and *PvuII* digests (data not shown).

A *BamHI* site is located to the right of the *PvuII* site in the  $\delta$ -globin gene. To determine whether this *BamHI* site is present in  $\delta\beta$ -1 DNA, double digests with *BamHI* were carried out. A *BamHI*/*PstI* digest of normal DNA produces fragments of 0.95 and 1.35 kilobases which hybridise the *Pst*  $\delta$  probe (Fig. 4b; a weakly hybridising  $\beta$ -gene fragment is also seen). The 0.95-kilobase fragment corresponds to the *PstI*/*BamHI* fragment to the left of the intragenic *BamHI* site, whereas the 1.35-kilobase fragment corresponds to the *BamHI*/*PstI* fragment to the right of this site. A *BamHI*/*PstI* digest of  $\delta\beta$ -1 DNA produces a 0.95 and a 3.3-kilobase fragment but no 1.35-kilobase fragment. Similarly, a *BamHI*/*EcoRI* digest of normal DNA produces fragments of 1.3, 1.0 and 1.75 kilobases (plus a  $\beta$ -gene fragment). The 1.3-kilobase fragment corresponds to the *EcoRI*/*BamHI* fragment to the left of the intragenic *BamHI* site; the 1.0-kilobase fragment to the *BamHI*/*EcoRI* fragment containing the  $\delta$ -globin intervening sequence; and the 1.75-kilobase fragment to the *EcoRI* fragment containing the 3'-portion of the  $\delta$ -globin gene. A *BamHI*/*EcoRI* digest of  $\delta\beta$ -1 DNA produces a 1.3-kilobase 5'- $\delta$ -gene fragment and a 1.9-kilobase fragment but not the 1.0- or 1.75-kilobase fragments of normal DNA. Together these results indicate that the restriction enzyme sites leftward from the intragenic *BamHI* site are present and that the *EcoRI* and *PstI* sites to the right of the *BamHI* site are missing in  $\delta\beta$ -1 DNA. This conclusion was confirmed by comparing *EcoRI*/*PvuII* or *PstI*/*PvuII* digests of normal and  $\delta\beta$ -1 DNAs (data not shown).

To further delineate the end point of the deletion, we compared the *HpaI* digestion patterns of normal and  $\delta\beta$ -1 DNA, as an *HpaI* site is located within the intervening sequence of the  $\delta$ -globin gene approximately 150 base pairs to the left of the *EcoRI* site (unpublished results). Digestion of normal DNA with *HpaI* produces fragments of 1.9 and 1.4 kilobases which hybridise with the  $\delta$ -globin probe (Fig. 4c; a large  $\beta$ -gene fragment is also observed). The 1.9-kilobase fragment contains the  $\delta$ -globin gene and the portion of the intervening sequence to the left of the *HpaI* site, and the 1.4-kilobase fragment contains sequences to the right of the *HpaI* site. Digestion of  $\delta\beta$ -1 DNA yields only one hybridising fragment of 2.1 kilobases, indicating that at least two of the three *HpaI* sites which delineate the 1.9- and 1.4-kilobase fragments in normal DNA are missing. Additional experiments using the RI H probe indicate that the *HpaI* site to the left of the  $\delta$ -globin gene in normal DNA is present in  $\delta\beta$ -1 DNA (data not shown). Therefore, the *HpaI* site near the right end of the intervening sequence is absent in  $\delta\beta$ -1 DNA.

In double digests with *BamHI* and either *PstI* or *EcoRI*, sequences to the right of the *BamHI* site hybridised to the *Pst*  $\delta$  probe. To form a stable hybrid in the conditions used, more than the 12 base pairs of coding sequence between the *BamHI* site and the beginning of the intervening sequence are required. Hence, at least some of the  $\delta$ -globin intervening sequence must be present in  $\delta\beta$ -1 DNA. Therefore, the deletion in  $\delta\beta$ -1 DNA





**Fig. 3** Hybridisation of normal and HPFH-1 DNA with the RI H fragment. Normal and HPFH-1 DNAs were hybridised as in Fig. 2 with *in vitro* labelled RI H DNA. The RI H fragment in H $\beta$ G1 DNA is located approximately 4 kilobases to the left of the  $\delta$ -globin gene (Fig. 1a) and is bounded on the right end by an *Eco*RI site present in genomic DNA and on the left end by an artificial *Eco*RI site created during the construction of the human DNA library<sup>19</sup>. The RI H fragment was isolated from H $\beta$ G1, subcloned into the *Eco*RI site of the plasmid pMB9 (ref. 29) and grown in *Escherichia coli*  $\chi$ 1776 (ref. 30). The RI H fragment was isolated from purified plasmid DNA of the subclone following *Eco*RI digestion. a, *Eco*RI digest; b, single and double enzyme digests using *Taq*I, *Xba*I and *Pst*I with *Eco*RI.

begins within the large intervening sequence of the  $\delta$ -globin gene and extends to at least 4 kilobases to the right of the  $\beta$ -globin gene (Fig. 1b).

### $\delta\beta$ -2 DNA

When  $\delta\beta$ -2 DNA<sup>10</sup> was digested with various restriction endonucleases, blotted and hybridised with the  $\beta$ -globin probe, no hybridisation was observed (ref. 10 and our unpublished results). Moreover, we detect no hybridisation with the RI H probe. The deletion in  $\delta\beta$ -2 DNA therefore extends through the  $\delta$ - and  $\beta$ -globin genes and beyond the RI H sequence. We next determined whether the deletion also covers restriction enzyme sites near the  $\gamma$ -globin genes. In *Bgl*II digests of normal DNA a 13-kilobase fragment containing both  $\gamma$ -globin genes was detected (Fig. 5a). However, in  $\delta\beta$ -2 DNA a 7-kilobase fragment was observed, indicating that one of the two *Bgl*II sites surrounding the  $\gamma$ -globin genes is deleted in  $\delta\beta$ -2 DNA. Digestion of normal DNA with *Eco*RI produces fragments of 7.2 and 2.7 kilobases which contain the 5'-portions of the  $\gamma$  and  $\delta$ -genes, respectively<sup>20</sup>. (Two fragments of 1.7 and 0.8 kilobases containing the 3' portions of these genes<sup>20</sup> are not efficiently detected with the pJW151  $\gamma$  cDNA probe.) Only the 7.2-kilobase 5'  $\gamma$  fragment was detected in  $\delta\beta$ -2 DNA. Thus, part or all of the  $\delta$ -gene is deleted in  $\delta\beta$ -2 DNA and sequences to the left of and including the intragenic *Eco*RI site in the  $\gamma$ -gene are present. This conclusion is consistent with the *Bam*HI hybridisation pattern. *Bam*HI digestion of normal DNA produces fragments of 15.5, 5.1 and 2.6 kilobases which hybridise to the  $\gamma$ -globin probe (Fig. 5b). These fragments contain, respectively, 3'  $\delta$ , 3'  $\gamma$  plus 5'  $\delta$ , and 5'  $\gamma$  sequences. Only the 5'  $\gamma$  fragment is observed in *Bam*HI digests of  $\delta\beta$ -2 DNA. The end point of the deletion within the

$\gamma$ -globin gene locus was more precisely mapped using the enzyme *Sac*I.

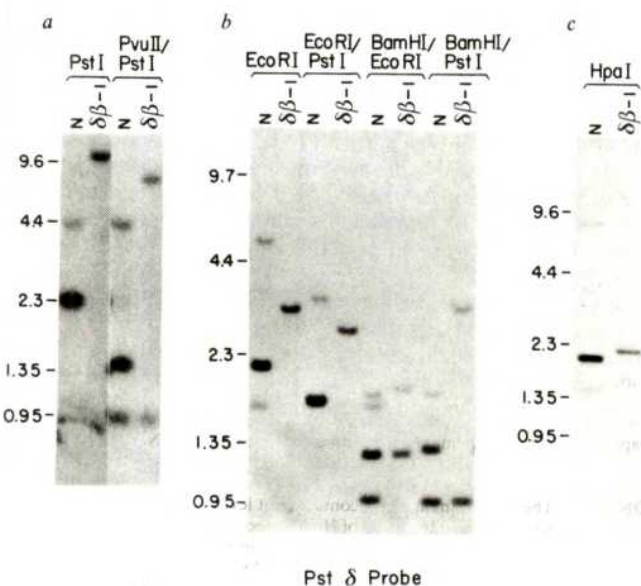
*Sac*I digestion of normal DNA produces fragments of 3.8 and 2.7 kilobases which hybridise the  $\gamma$ -globin probe (Fig. 5b). These fragments contain the  $\delta$ - and  $\gamma$ -gene sequences, respectively. *Sac*I digestion of  $\delta\beta$ -2 DNA produces only the 2.7-kilobase  $\gamma$  fragment, indicating that the *Sac*I site to the right of the  $\gamma$ -intragenic *Eco*RI site is present in  $\delta\beta$ -2 DNA. No additional fragments were observed, again implying that part or all of the  $\delta$ -gene is deleted.

To determine whether the *Eco*RI site to the right of the  $\gamma$ -gene is present in  $\delta\beta$ -2 DNA, a cloned genomic DNA fragment containing 3'  $\gamma$  sequences was used as a probe (*Xba*  $\gamma$ ; Fig. 1). Figure 5c shows the *Eco*RI lanes from the filter of Fig. 5a reprobed with the *Xba*  $\gamma$  fragment. With the *Xba*  $\gamma$  probe the 1.7-kilobase 3'  $\gamma$  *Eco*RI fragment of normal DNA is detected (compare Fig. 5a and c). This fragment is missing in  $\delta\beta$ -2 DNA, indicating that the deletion removes the *Eco*RI site to the right of the  $\gamma$ -gene. Thus, the deletion in  $\delta\beta$ -2 DNA begins in the region between the *Sac*I site within the  $\gamma$ -gene<sup>32</sup> and the *Eco*RI site to the right of the gene and extends through the  $\delta$ -gene. The same (or possibly a second) deletion extends through the RI H,  $\delta$ - and  $\beta$ -globin sequence (Fig. 1b).

### Physical linkage of $\gamma$ -, $\delta$ - and $\beta$ -globin genes

Linkage between the fetal and adult globin genes was suggested by structural analysis of Hb Kenya<sup>17</sup>, a fusion protein consisting of  $\delta$ -globin N-terminal and  $\beta$ -globin C-terminal sequences. However, direct physical linkage has not been demonstrated. We have used cloned genomic DNA hybridisation probes and the deletion map of HPFH-1 DNA (Fig. 1b) to demonstrate unambiguously physical linkage between the  $\delta$ - and  $\beta$ -globin genes.

Initially, we identified large restriction enzyme fragments in normal DNA which hybridise the  $\gamma$ -globin and RI H probes. As shown in Fig. 6a the RI H probe hybridises with a 15.5-kilobase *Bam*HI fragment and a 14.0-kilobase *Hpa*I fragment in normal



**Fig. 4** Hybridisation of normal and  $\delta\beta$ -1 DNAs with the  $\delta$ -globin *Pst*I fragment. Normal and  $\delta\beta$ -1 DNAs were hybridised as described in the legend to Fig. 2 with *in vitro* labelled *Pst*  $\delta$  DNA. The *Pst*  $\delta$  fragment of H $\beta$ G1 DNA contains the  $\delta$ -globin gene and some surrounding sequences (Fig. 1a). The *Pst*  $\delta$  fragment was isolated from H $\beta$ G1, subcloned into the *Pst*I site of the plasmid pBR322 (ref. 31) and grown in *E. coli*  $\chi$ 1776 (ref. 30). The *Pst*  $\delta$  fragment was isolated from the purified plasmid DNA of the subclone following *Pst*I digestion. a, *Pst*I/*Pvu*II double digest; b, double digests using *Eco*RI, *Pst*I and *Bam*HI; c, *Hpa*I digest.



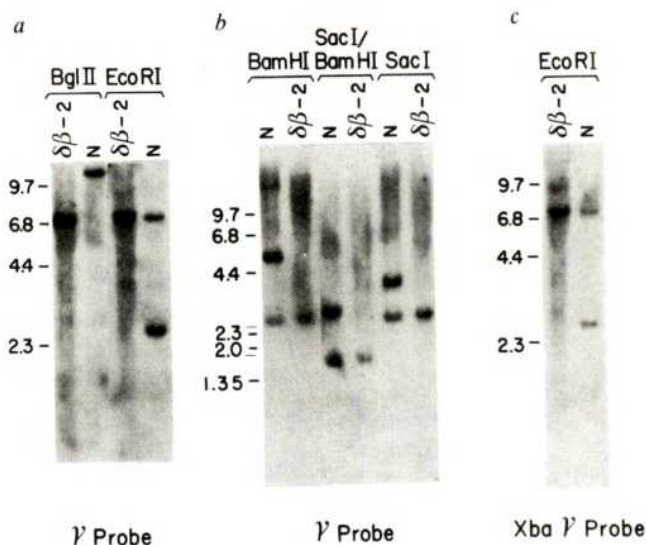
DNA. Fragments of these sizes are also detected using the pJW151  $\gamma$ -gene probe (Fig. 6b) or a probe specific for the 3'-end of the  $\gamma$ -genes (Fig. 6c). The 15.5-kilobase fragment corresponds to a 15-kilobase fragment which was previously shown to contain 3'- $\gamma$ -sequences<sup>20</sup>. Our mapping experiments (unpublished) indicate that the 14.0-kilobase *Hpa*I fragment also contains 3'- $\gamma$ -sequences. These results indicate that RI H and 3'- $\gamma$ -sequences are present on fragments of identical size in *Bam*HI or *Hpa*I digests. Thus, the 3' end of the  $\gamma$ -gene seems to be linked to the RI H fragment. Definitive evidence for this linkage relationship was obtained by comparing the sizes of the *Bam*HI and *Hpa*I fragments in normal and HPFH-1 DNA using RI H and  $\gamma$ -globin probes. If the linkage arrangement proposed above is correct, the deletion in HPFH-1 should alter the size of the *Bam*HI and *Hpa*I fragments detected by both the RI H and  $\gamma$ -probes. As predicted, the 15.5-kilobase *Bam*HI and 14.0-kilobase *Hpa*I fragments of normal DNA are not seen in HPFH-1 DNA, whereas 13.0-kilobase *Bam*HI and 13.0-kilobase *Hpa*I fragments are observed (Fig. 6a-c). Thus, the deletion in HPFH-1 DNA, which terminates near the RI H fragment, has an identical effect on the size of restriction enzyme fragments detected by either the RI H or  $\gamma$ -gene probes. These results definitely establish physical linkage between the 3'- $\gamma$ -gene and the RI H fragment.

We have previously shown that the RI H fragment is linked to the 5' end of the  $\delta$ -globin gene in cloned genomic DNA<sup>19</sup>. The linkage of the 3'- $\gamma$ -gene with the RI H fragment described here, and the linkage of the RI H fragment with the 5'- $\delta$ -gene previously described provide unambiguous proof for the linkage arrangement shown in Fig. 1a. Consistent with this conclusion, we have observed that a 15.5-kilobase *Bam*HI fragment also hybridises with the  $\beta$ -globin probe (Fig. 6d) in conditions in which cross-hybridisation of  $\gamma$ -gene sequences with the  $\beta$ -globin probe is not observed (compare Fig. 6b and d). The  $\beta$ -like globin genes are therefore arranged in the order: 5'- $\gamma$ - $\gamma$ - $\delta$ - $\beta$ 3'.

## Discussion

We have used the detailed map of restriction endonuclease cleavage sites within and surrounding the  $\delta$ - and  $\beta$ -globin genes to locate the end points of deletions in the DNA from individuals with  $\delta\beta$ -thalassaemia and HPFH we have designated  $\delta\beta$ -1 and HPFH-1 and 2. The deletions in HPFH-1 and 2 are indistinguishable. In addition, the haematological findings in the two cases are virtually identical<sup>3,4,11,12</sup>. Although there is no known family relationship between the HPFH-1 and 2 individuals, it is possible that the HPFH chromosomes in the two cases had the same ultimate origin. Alternatively, it is possible that the end point of the deletions represents a mutational hot spot so that the two deletions were in fact the products of independent events.

The haematological findings in the  $\delta\beta$ -1 and HPFH-1 and 2 cases reveal several significant differences<sup>3,4,11,12,22</sup>. In  $\delta\beta$ -1, characteristics of thalassaemia which include anaemia and hypochromic, microcytic red cells were observed. In addition, analysis of heterozygous relatives indicated that 10% of their haemoglobin was HbF containing both  $\gamma$ - and  $\delta$ -polypeptide chains and the cellular distribution was heterogeneous (30–40% of the cells stain for HbF). In HPFH-1 and 2 none of the characteristics of thalassaemia was observed. In heterozygous relatives approximately 20–30% of the haemoglobin was HbF (both  $\gamma$ - and  $\delta$ -) and the cellular distribution was homogeneous (100% of the cells stain for HbF). The high levels of HbF found in  $\delta\beta$ -thalassaemia heterozygotes may be due in part to the increased proliferation or selective survival of a small population (1%) of 'F-cells' found in the blood of normal individuals<sup>4</sup>. This is not a general phenomenon, however, as in heterozygous  $\beta^0$ -thalassaemia, which in most cases is a non-deletion type of  $\beta$ -globin deficiency<sup>9</sup>, very little HbF is found in adults. In fact, homozygous  $\beta^0$ -thalassaemia is a much more severe type of anaemia than homozygous  $\delta\beta$ -thalassaemia.



**Fig. 5** Hybridisation of normal and  $\delta\beta$ -2 DNAs with the cloned  $\gamma$ -globin cDNA plasmid and with the Xba  $\gamma$  fragment. Normal and  $\delta\beta$ -2 DNAs were hybridised as in Fig. 2 with the *in vitro* labelled *Hha*I fragment of pJW151 DNA<sup>25</sup> or the Xba  $\gamma$  fragment. The Xba  $\gamma$  fragment was isolated following *Xba*I digestion of H $\gamma$ G1, a clone of human DNA containing the  $\gamma$ - and  $\delta$ -globin genes (unpublished results). The Xba  $\gamma$  fragment contains the DNA sequences between *Xba*I sites located at similar positions in the intervening sequence of the  $\gamma$ - and  $\delta$ -genes (Fig. 1a). a, *Bgl*II, *Eco*RI digests using the  $\gamma$  probe; b, *Bam*HI, *Sac*I single and double digests using the  $\gamma$  probe; c, *Eco*RI lanes from a reprobed with the Xba  $\gamma$  fragment.

Thus, there seems to be a correlation between the extent of the deletion of the  $\delta$ - and  $\beta$ -globin genes and the level of  $\gamma$ -globin gene expression in adults, suggesting that sequences involved in the suppression of  $\gamma$ -gene expression are located in the  $\delta$ - $\beta$ -globin gene region. This deletion model for  $\gamma$ -gene expression in adults was first elaborated by Huisman *et al.*<sup>7</sup>. A comparison of the deletions in HPFH-1 and 2 and  $\delta\beta$ -1 suggests that one such regulatory sequence may be located within a 4-kilobase region on the 5' side of the  $\delta$ -globin gene. An alternative possibility is that HPFH-1 and 2 are double mutants, the second lesion affecting a negative regulatory sequence located in the  $\gamma$ -globin gene region<sup>3</sup>. Our data rule out the presence of a large deletion of sequences surrounding the  $\gamma$ -genes, but a small deletion or point mutation would not have been detected. Although the occurrence of a second mutation in all the different types of HPFH described seems unlikely, the double mutation hypothesis remains a formal possibility. Furthermore, the possibility that the HPFH phenotype results from the deletion of sequences 3' to the  $\beta$ -globin gene cannot be ruled out by our data since we have not located the rightward end points of the various deletions. However, the fact that individuals with Hb Kenya express the HPFH phenotype argues against this possibility. In Hb Kenya the region between  $\gamma$  and  $\delta$  genes is thought to be missing but the sequences which lie to the 3' side of the  $\beta$  gene are presumed to be intact<sup>7</sup>.

If there are regulatory sequences surrounding the  $\delta$ - and  $\beta$ -globin genes, what is the nature of their action? Analysis of heterozygous cases of HPFH in which only one of the  $\gamma$ -globin chains is expressed in adults have led to the suggestion that the HPFH deletion affects  $\gamma$ -gene expression *in cis*. For example, in heterozygotes of  $\gamma$ -HPFH, only the  $\gamma$ -polypeptide is detected in adults even though the  $\delta$ -chain on the normal chromosome is functional (the  $\delta$ -polypeptide is detected at birth in individuals with  $\gamma$ -HPFH but disappears during postnatal development)<sup>33</sup>. If the regulatory sequence acted *in trans*, sequences on the normal chromosome would suppress the expression of both  $\gamma$ -genes in the heterozygote. An analogous argument can be made for the heterozygous  $\gamma$ - $\delta$ -types of HPFH. If, indeed, a



*cis*-acting regulatory sequence is present in  $\delta\beta$ -1 but absent in HPFH-1 and 2 DNAs, its location, 12 kilobases to the 3' side of the  $\gamma$ -gene (Fig. 1), is unprecedented. We know of no example of a 3'-*cis* acting control sequence in either a prokaryotic or eukaryotic gene system. However, it is reasonable to suppose that sequences which lie to the 3' side of a gene could function in globin RNA processing or splicing<sup>34</sup>, in the activation of a chromosome region similar to the phenomenon of puffing in *Drosophila* polytene chromosomes<sup>35</sup>, or in an immunoglobulin-type rearrangement of DNA sequences during development<sup>36</sup>. The availability of cloned segments of DNA covering the entire  $\beta$ -like globin gene locus will make it feasible to test these possibilities.

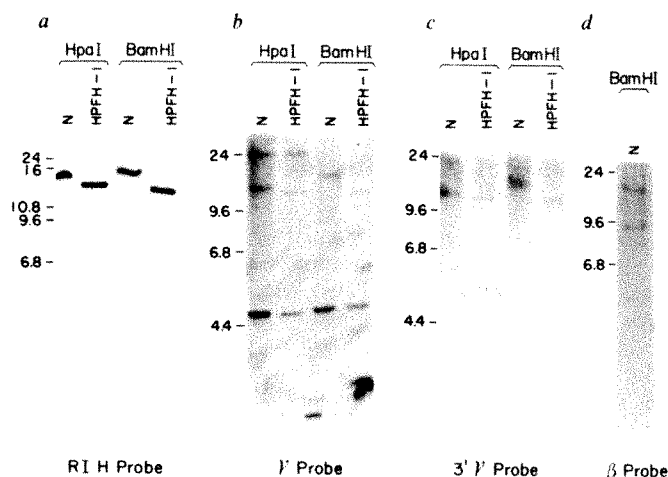
The correlation of the haematological data of the  $\delta\beta$ -2 case with the deletion map of Fig. 1 seems to argue against the existence of a negative regulatory sequence on the 5' end of the  $\delta$ -globin gene. Clinical data reveal a moderately severe anaemia which includes marked disorders in red cell morphology. The heterozygous parents of  $\delta\beta$ -2, who are first cousins, have 10–13% HbF which is non-uniformly distributed. Although peptide analysis of the HbF in  $\delta\beta$ -2 has not been reported, our data show that only the  $\gamma$ -chain could be produced. Thus,  $\delta\beta$ -2 seems to be an example of  $\gamma\delta$ -thalassaemia. However, the deletion model would predict an HPFH phenotype as the putative regulatory sequence located near the 5' end of the  $\delta$ -globin gene is missing in  $\delta\beta$ -2 DNA. This discrepancy might be explained by the fact that the deletion in  $\delta\beta$ -2 DNA extends through the  $\gamma$ -gene and into the region between the  $\gamma$  and  $\delta$ -genes. Only one case each of homozygous  $\gamma$ -HPFH<sup>37,38</sup> and  $\gamma\delta$ -thalassaemia<sup>10</sup> ( $\delta\beta$ -2) has been reported. These individuals were designated HPFH or  $\delta\beta$ -thalassaemia on the basis of analysis of heterozygous relatives even though both homozygotes displayed many of the characteristics of  $\delta\beta$ -thalassaemia. Altay *et al.* have previously discussed<sup>8</sup> the difficulty in discriminating between  $\gamma$ -types of HPFH and  $\delta\beta$ -thalassaemia. Even if  $\gamma$ -gene suppression was eliminated in  $\gamma$ -HPFH, a chain

imbalance resulting from the deletion of the  $\gamma$ -gene and surrounding sequences would result in thalassaemia traits. Thus, it may not be valid to compare  $\gamma$ - and  $\gamma\delta$ -types of HPFH. Until further information regarding the locations of deletions in cases of  $\gamma$ -HPFH and  $\delta\beta$ -thalassaemia can be obtained, the significance of the  $\delta\beta$ -2 result with regard to the deletion model cannot be assessed.

The experiments described here represent an initial step towards localising sequences which are involved in controlling the switch from fetal to adult  $\beta$ -like globin gene expression in man. The construction of a detailed linkage map of the  $\beta$ -like globin genes and the use of cloned hybridisation probes which span the entire region have established the feasibility of mapping deletions in human DNA. Hopefully, a similar analysis of additional cases of HPFH and  $\delta\beta$ -thalassaemia will ultimately lead to the identification of sequences which are involved in controlling these developmentally regulated genes.

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**Fig. 6** Hybridisation of normal and HPFH-1 DNA with cloned  $\beta$ - and  $\gamma$ -globin cDNA and the RI H fragment—linkage of fetal and adult  $\beta$ -like globin genes. Normal and HPFH-1 DNAs were hybridised as described in the legend to Fig. 2 with the hybridisation probes indicated below each panel. 5'- and 3'- $\gamma$ -globin specific DNA was prepared by digesting pJW151 DNA<sup>25</sup> with *Hha*I and *Bam*HI and purifying the fragments containing  $\gamma$ -globin sequences 5' and 3' to the intragenic *Bam*HI site. The 3'- $\gamma$ -globin specific DNA was labelled *in vitro* by nick translation<sup>27</sup>. A threefold excess of unlabelled 5'- $\gamma$ -globin specific DNA was included in the hybridisation as competing DNA. The remaining hybridisation probes have been described (see Figs. 2 and 3 legends). Multimeric forms of  $\Phi$ X174 DNA were included in the gel as size markers<sup>19</sup>. *a*, *Hpa*I and *Bam*HI digests using the RI H probe; *b*, *Hpa*I and *Bam*HI digests using the  $\gamma$  probe; *c*, *Hpa*I and *Bam*HI digests using the 3'- $\gamma$  probe; *d*, *Bam*HI digest using the  $\beta$  probe.

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# Biosynthesis of the major human red cell sialoglycoprotein, glycophorin A, in a continuous cell line

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*During biosynthesis of glycophorin A in K562 cells a precursor is rapidly transferred through the endoplasmic reticulum membrane with the COOH-terminal remaining in the cytoplasm. This is glycosylated within the cell and appears at the cell surface after about 30 min. The biosynthetic pathway resembles that described for viral membrane glycoproteins.*

MOST, if not all, surface proteins of mammalian cells are glycoproteins<sup>1,2</sup>. Their peptide portions often span the lipid bilayer membrane<sup>3,4</sup> and their carbohydrate is exposed to the external milieu<sup>5-7</sup>. The biosynthetic assembly of the peptide and sugar portions and the mechanisms leading to the appropriate membrane disposition of these proteins raise intriguing questions.

Studies on virus-coded proteins have provided us with most of our more detailed knowledge of integral membrane protein biosynthesis. The polypeptides are synthesised on membrane-bound ribosomes and transferred as nascent chains through the endoplasmic reticulum membrane; glycosylation takes place on the luminal side<sup>8-10</sup>. The attachment of the core portions of the oligosaccharides may occur, through lipid-linked intermediates, even before the polypeptide chains have been completely synthesised. As the virus proteins use the host cell machinery for their biosynthesis, similar mechanisms may be anticipated for mammalian surface proteins.

Most of our information on cell membrane structure is derived from studies on the easily available human erythrocyte membrane<sup>2,13,14</sup>. Its major sialic acid-rich glycoprotein, glycophorin A (refs 15, 16), is the best characterised integral membrane protein. Glycophorin A spans the membrane with its NH<sub>2</sub>-terminus on the outside and its COOH-terminus in the cytoplasm<sup>3,4</sup>, and has a molecular weight (MW) of 31,000. On polyacrylamide gel electrophoresis in the presence of SDS it shows a higher apparent MW because of its 60% carbohydrate content<sup>3,17</sup>. Determination of its amino acid sequence<sup>18,19</sup> indicates that it has 15 serine/threonine-linked oligosaccharides and one asparagine-linked complex oligosaccharide on the external surface of the erythrocyte.

We have produced specific anti-glycophorin A antiserum<sup>20</sup> by immunising rabbits with a crude preparation of glycophorin followed by absorption with En(a-) red cell membranes, which lack glycophorin<sup>16,21-23</sup>. The availability of this specific reagent for glycophorin A and our extensive knowledge of the molecular structure of this glycoprotein provide a potentially useful system for elucidating the pathways involved in mammalian plasma membrane glycoprotein biosynthesis.

We have previously observed that the human continuous leukaemia cell line K562 (ref. 24) is erythroid, and synthesises and expresses glycophorin A on its surface<sup>25,26</sup>. In this report we

outline the biosynthesis of glycophorin A from a precursor form, its insertion into the endoplasmic reticulum membrane, its subsequent glycosylation and its migration to the plasma membrane.

## Identification of a precursor for glycophorin A

For studies on the biosynthesis of glycophorin A, K562 cells were labelled with <sup>35</sup>S-methionine for 5 min and chased with medium containing non-radioactive methionine. The labelled cells were solubilised in buffer containing Triton X-100 and immune precipitation was carried out with anti-glycophorin A antiserum or control serum and protein A-containing staphylococci<sup>20</sup>. The precipitates were analysed by polyacrylamide slab gel electrophoresis in the presence of SDS followed by fluorography. After 5 min of labelling a single protein was specifically precipitated. It had an apparent MW of 37,000 and was designated GP<sub>a</sub> (Fig. 1a, B). The use of internal labelling meant that a high background radioactivity could not be avoided and therefore the appearance of the specifically precipitated protein could only be followed by using controls with preimmunisation serum. When chased for 10 min (Fig. 1a, D) the final glycophorin A molecule (designated GP<sub>c</sub>), with an apparent MW of 39,000, became visible, and its concentration relative to that of GP<sub>a</sub> increased during chase for 25–60 min (Fig. 1a, F–J).

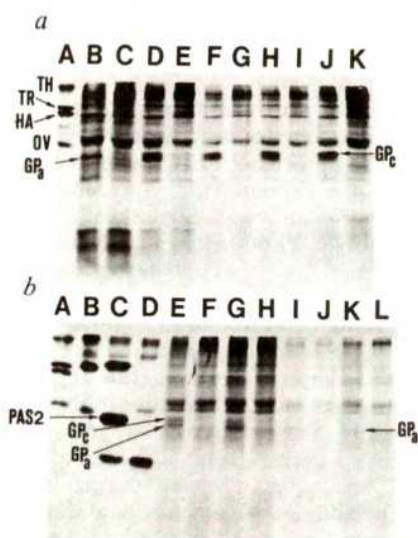
## Appearance of glycophorin A at the cell surface

As surface-exposed glycophorin A can be cleaved by trypsin<sup>15,26</sup> the time course of its externalisation was followed by trypsin treatment of labelled intact cells. After labelling for 5 min followed by chase for 10 min, neither the precursor protein, GP<sub>a</sub>, nor the completed glycophorin A (GP<sub>c</sub>) had reached the cell surface, as indicated by their insensitivity to trypsinisation (Fig. 1b, E, G). After 25 and 45 min chase no GP<sub>c</sub> was seen in trypsin-treated cells, but GP<sub>a</sub> was still detected (Fig. 1b, I, K). In the absence of trypsin treatment the GP<sub>c</sub> band was stronger than the GP<sub>a</sub> band at these time points (compare Fig. 1a, F, H). This shows that after 25 min glycophorin A had appeared at the cell surface but that GP<sub>a</sub> had not become exposed.

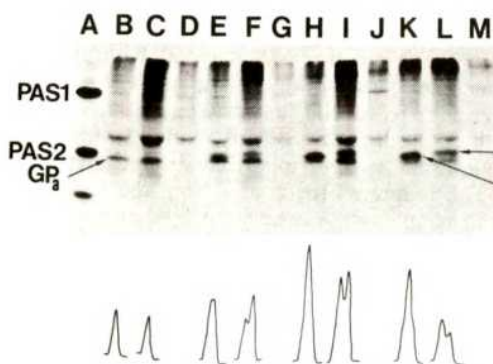
## Glycophorin A precursor is incompletely glycosylated

We followed the sequence of glycosylation using lectin columns of known specificity. The glycophorin A precursor, GP<sub>a</sub>, already contained glucose/mannose-like residues because it bound to lentil lectin-Sepharose and could be eluted with  $\alpha$ -methylmannoside (Fig. 2B). However, the following results indicate that GP<sub>a</sub> does not contain significant amounts of galactose or





**Fig. 1** *a*, Fluorography of a polyacrylamide slab gel of immune precipitates obtained from pulse-chased  $^{35}\text{S}$ -methionine-labelled K562 cells with anti-glycophorin A antiserum and control serum. K562 cells ( $80 \times 10^6$ ) were grown in RPMI-1640 culture medium (GIBCO) containing 10% newborn calf serum. After washing three times with 0.15 M NaCl and 0.01 M sodium phosphate, pH 7.4 (PBS), at  $37^\circ\text{C}$ , the cells were suspended in 5 ml of methionine-free Eagle's minimal essential medium (MEM) containing 0.2% bovine serum albumin (Sigma). The cells were incubated with gentle shaking in a  $\text{CO}_2$ -atmosphere for 30 min at  $37^\circ\text{C}$  and 1.2 mCi of  $^{35}\text{S}$ -methionine (Radiochemical Centre, 1,130 Ci mmol $^{-1}$ ) added. After 5 min 1 ml of the suspension was withdrawn to ice-cold PBS and the cells immediately washed three times with PBS at  $0^\circ\text{C}$ . The rest of the pulse-labelled cells were rapidly washed at  $37^\circ\text{C}$  with MEM containing a 50-fold excess of cold methionine and suspended in 4 ml of this medium. After incubation for 10, 25, 45 and 60 min 1-ml aliquots were removed and the cells washed at  $0^\circ\text{C}$  as above. All washed cell samples were then suspended in 4 ml of PBS at  $0^\circ\text{C}$  and 1 ml from each taken for trypsinisation (see *b* below). The rest of the samples were centrifuged and the cells dissolved at  $0^\circ\text{C}$  in PBS containing 1% Triton X-100, 1% ethanol and 2 mM phenylmethylsulphonylfluoride (Sigma) as a protease inhibitor (buffer A). Aliquots of the Triton X-100 extracts were taken for immune precipitation experiments using 5  $\mu\text{l}$  anti-glycophorin A antiserum or control serum and the *Staphylococcus aureus* protein A technique as described in detail previously $^{20,26}$ . The immune precipitates were studied by polyacrylamide slab gel electrophoresis in the presence of SDS $^{34}$  using a 12% acrylamide concentration in the separating gel. The treatment of the slab gels for fluorography $^{35}$  and the  $^{14}\text{C}$ -labelled $^{36}$  standard proteins have been described earlier $^{27}$ . A,  $^{14}\text{C}$ -labelled standard proteins: TH = thyroglobulin; TR = transferrin; HA = human albumin; OV = ovalbumin; B, immune precipitate obtained with anti-glycophorin A antiserum from K562 cells labelled for 5 min with  $^{35}\text{S}$ -methionine; C, with preimmune serum; D, immune precipitate obtained with antiserum from cells labelled for 5 min with  $^{35}\text{S}$ -methionine followed by chase for 10 min; E, with preimmune serum; F, immune precipitate obtained with antiserum from cells after 25 min chase; G, with preimmune serum; H, immune precipitate obtained with antiserum from cells after 45 min chase; I, with preimmune serum; J, immune precipitate obtained with antiserum from cells after 60 min chase; K, with preimmune serum. *b*, Fluorography of a polyacrylamide slab gel of labelled erythrocyte membranes and immune precipitates obtained from trypsin-treated K562 cells labelled with  $^{35}\text{S}$ -methionine. Part of the  $^{35}\text{S}$ -methionine-labelled cells obtained from the experiment described for *a* were treated with 0.1 mg ml $^{-1}$  trypsin (Merck, Darmstadt) for 10 min at  $37^\circ\text{C}$ . The samples were then immediately washed with PBS at  $0^\circ\text{C}$  and solubilised in buffer A. Immune precipitations and polyacrylamide slab gel electrophoresis were carried out as described in *a*. A,  $^{14}\text{C}$ -labelled standard proteins (same as in *a*); B, surface glycoprotein pattern of erythrocyte membranes labelled with  $^3\text{H}$  after treatment of erythrocytes with neuraminidase and galactose oxidase followed by  $\text{NaB}^3\text{H}_4$  (ref. 6); C, surface glycoprotein pattern of erythrocyte membranes obtained after labelling erythrocytes by the periodate/ $\text{NaB}^3\text{H}_4$  method $^{27}$ ; PAS 2 = predominantly glycophorin A monomer; D, surface glycoprotein pattern of periodate/ $\text{NaB}^3\text{H}_4$ -labelled membranes after treatment of labelled intact erythrocytes with 0.1 mg ml $^{-1}$  trypsin for 10 min at  $37^\circ\text{C}$ ; E, immune precipitate obtained with anti-glycophorin A antiserum from trypsinised K562 cells that had been labelled with  $^{35}\text{S}$ -methionine for 5 min; F, with preimmune serum; G, immune precipitate obtained with antiserum from cells labelled for 5 min with  $^{35}\text{S}$ -methionine followed by chase for 10 min; H, with preimmune serum; I, immune precipitate with antiserum from cells after 25 min chase; J, with preimmune serum; K, immune precipitate with antiserum from cells after 45 min chase; L, with preimmune serum.



**Fig. 2** Fluorography of a polyacrylamide slab gel of glycophorin molecules bound to lentil lectin-Sepharose and the effect of neuraminidase on their electrophoretic mobilities. K562 cells were labelled with  $^{35}\text{S}$ -methionine as described in Fig. 1*a* legend, washed at  $0^\circ\text{C}$  with PBS, solubilised in buffer A and applied to lentil lectin-Sepharose columns. *Lens culinaris* lectin was prepared by affinity chromatography $^{38}$  and coupled to CNBr-activated Sepharose 4B as described in detail previously $^{37}$ . The material eluted with 0.1 M  $\alpha$ -methyl mannoside (Calbiochem) was pooled and concentrated by vacuum dialysis to 3 ml. To 1-ml aliquots from each sample was added 0.3 ml of Dulbecco's PBS containing  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  and 100 units of *Vibrio cholerae* neuraminidase (Behringwerke). The neuraminidase preparation did not contain proteolytic activity when assayed as described previously $^6$ . Two other identical aliquots did not receive neuraminidase. All samples were incubated for 10 min at  $37^\circ\text{C}$ . After incubation one control sample and the neuraminidase-treated sample were incubated with 5  $\mu\text{l}$  anti-glycophorin A antiserum and the third tube with preimmune serum followed by protein A-containing staphylococci. The immune precipitates were then analysed by polyacrylamide slab gel electrophoresis as described above. A, surface glycoprotein pattern of periodate/ $\text{NaB}^3\text{H}_4$ -labelled erythrocyte membranes; B, immune precipitate obtained with anti-glycophorin A antiserum from the glycoprotein fraction of K562 cells labelled for 5 min with  $^{35}\text{S}$ -methionine; C, immune precipitate with antiserum from the neuraminidase-treated glycoprotein fraction of cells labelled for 5 min; D, with preimmune serum; E, immune precipitate obtained with antiserum from the glycoprotein fraction of cells labelled for 5 min followed by 10 min chase; F, immune precipitate with antiserum from the neuraminidase-treated glycoprotein fraction of cells labelled for 5 min followed by 10 min chase; G, with preimmune serum; H, immune precipitate obtained with antiserum after 25 min chase; I, immune precipitate with antiserum of the 25-min chase sample treated with neuraminidase; J, with preimmune serum; K, immune precipitate obtained with antiserum after 60 min chase; L, immune precipitate with antiserum of the 60-min chase sample treated with neuraminidase; M, with preimmune serum. The corresponding scanning patterns of the glycophorin regions are shown below and were obtained with a Joyce-Loebl Chromoscan apparatus.

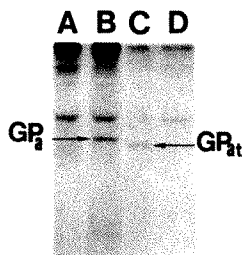
sialic acids. First, it did not bind to *Ricinus communis* lectin-Sepharose columns specific for  $\beta$ -D-galactose (data not shown). Second, as it is known that neuraminidase treatment of erythrocyte glycophorin A reduces its mobility on SDS-gel electrophoresis this phenomenon was used to determine the presence of sialic acids $^{27}$ . Part of the  $^{35}\text{S}$ -methionine-labelled eluates from the lentil lectin columns were treated with neuraminidase before immune precipitation.  $\text{GP}_a$  mobility on SDS-gel electrophoresis did not change after neuraminidase treatment, but instead of final glycophorin A, a molecule, designated  $\text{GP}_b$ , with an apparent MW of 41,000, was detected. This demonstrates that  $\text{GP}_c$  contains sialic acids.  $\text{GP}_c$  could be adsorbed not only to lentil lectin-Sepharose but also to *R. communis* lectin-Sepharose columns, indicating that it contains terminal  $\beta$ -D-galactosyl residues. This experiment also indicates that at this stage  $\text{GP}_a$  does not contain the penultimate galactosyl residues because its mobility would then have corresponded to that of  $\text{GP}_b$ .

### The $\text{NH}_2$ -terminal portion of glycophorin A precursor is rapidly incorporated into microsomes

The incorporation of the glycophorin A precursor into endoplasmic reticulum was studied by labelling K562 cells for 5 min with  $^{35}\text{S}$ -methionine. The labelled cells were then immediately



cooled to 0 °C, homogenised and a microsomal fraction prepared. Half of this fraction was treated with trypsin. The untreated and the trypsin-treated samples were solubilised in buffer A (see Fig. 1a legend) and passed through lentil lectin columns; the glycoproteins were eluted with  $\alpha$ -methylmannoside and immune precipitated with anti-glycophorin A antiserum. The immune precipitates from the trypsin-treated and untreated samples were electrophoresed on parallel slots of a polyacrylamide slab gel. Complete, 37,000 MW, glycophorin precursor, GP<sub>a</sub>, was recovered from untreated microsomes (Fig. 3B) but the molecule (GP<sub>at</sub>) obtained from the trypsin-treated microsomes had an apparent MW of 34,000 (Fig. 3C). This shows that a fragment of approximate MW 3,000 could be cleaved off by trypsin at this stage.



**Fig. 3** Fluorography patterns of immune precipitates from K562 microsomes analysed by polyacrylamide slab gel electrophoresis. K562 cells ( $170 \times 10^6$ ) were labelled for 5 min with  $^{35}\text{S}$ -methionine as described in Fig. 1a legend. They were then rapidly washed three times with PBS at 0 °C and suspended for 10 min in 15 ml of 10 mM KCl, 10 mM Tris and 1.5 mM  $\text{MgCl}_2$ , pH 7.4, at 0 °C. The cells were then homogenised in a tight-fitting Dounce homogeniser with 20 strokes and the nuclei removed by centrifugation at 1,000g for 10 min. The supernatant was centrifuged at 50,000g for 90 min at 4 °C in a Spinco rotor 30 and the microsomal pellet obtained suspended in 1 ml of Dulbecco's PBS. Half of this material was incubated with 0.01 mg ml<sup>-1</sup> trypsin for 10 min at 37 °C. The control sample was handled in the same way except that trypsin was omitted. After incubation 0.5 mg of soybean trypsin inhibitor (Sigma) was added to both samples. These were then dissolved in buffer A, both divided into two and immune precipitated with either 5  $\mu\text{l}$  anti-glycophorin A antiserum or preimmune serum. The immune precipitates were then analysed on a 12% polyacrylamide slab gel. A, Control microsomes + preimmune serum; B, control microsomes + anti-glycophorin A antiserum; C, trypsin-treated microsomes + anti-glycophorin A antiserum; D, trypsin-treated microsomes + preimmune serum. Note the decrease in the apparent MW of trypsin-treated GP<sub>a</sub>.

## Discussion

We recently showed that glycophorin A is expressed not only on mature red cells but also on nucleated precursor cells in human bone marrow<sup>20</sup>. This finding prompted us to look for cell lines synthesising glycophorin A. Our observation that the human continuous cell line K562 is erythroid<sup>25</sup> was the basis for the present experiments. The cells expressed  $10^6$  copies per cell of a surface glycoprotein which is indistinguishable from erythrocyte glycophorin A. The K562 protein has an apparent MW identical to that of glycophorin A from red cells, it dimerises easily—a characteristic of the erythrocyte protein—it reacts with anti-glycophorin A antiserum, contains MN blood group activity, gives rise to CNBr fragments and contains glycopeptides/oligosaccharides which closely resemble those of glycophorin A (ref. 26). Furthermore, treatment of K562 cells with sodium butyrate induces erythroid differentiation including haemoglobin synthesis and the generation of erythrocyte-like particles<sup>28</sup>.

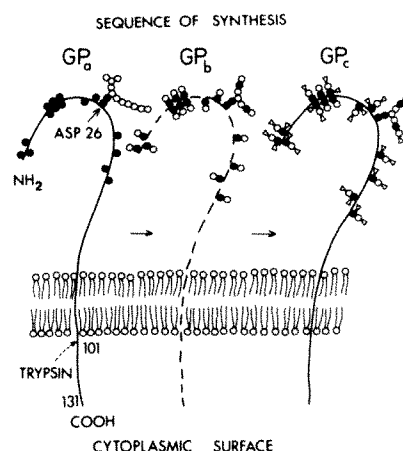
A schematic model of the way in which we consider the biosynthesis of glycophorin A to occur is shown in Fig. 4. Three forms of glycophorin A can be recognised, GP<sub>a</sub>, GP<sub>b</sub> and GP<sub>c</sub>. GP<sub>c</sub> is the final glycophorin A molecule which is synthesised completely within the cell and is present at the cell surface after 35 min of pulse labelling. (This includes the 10 min required for trypsinisation.) This glycoprotein contains the terminal sialic acid residues and the penultimate galactosyl residues<sup>26</sup> and has

an apparent MW of 39,000 on 12% polyacrylamide gels. The presence of sialic acids gives it a higher electrophoretic mobility in the presence of SDS than after removal of these residues<sup>27</sup> (Fig. 2). GP<sub>b</sub>, which can be obtained from GP<sub>c</sub> only after neuraminidase treatment, has an apparent MW of 41,000.

GP<sub>a</sub> is obviously a precursor of glycophorin A. It reacts with anti-glycophorin A antiserum and the precursor/product relationship is evident from the pulse-chase experiments (Fig. 1a). It has an apparent MW of 37,000, does not adsorb to *R. communis* lectin-Sepharose and does not contain sialic acids (Fig. 2B,C). The 4,000 MW difference between desialylated glycophorin A (GP<sub>b</sub>) and GP<sub>a</sub> should correspond to approximately 20 monosaccharides. This cannot be accounted for solely by the completion of the complex oligosaccharide at Asn 26 but must involve the addition of galactosyl groups to the 15 alkali-labile chains. Apparently, the sialic acids are then rapidly added because a precursor form corresponding to desialylated glycophorin A (GP<sub>b</sub>) is never observed during the pulse-chase experiments. The addition of galactosyl and sialic acid residues is known to occur late in the biosynthesis of glycoproteins, mainly in Golgi membranes<sup>29</sup>.

GP<sub>a</sub> is found after 5 min of labelling in microsomes when the carbohydrate is protected from the action of trypsin (Fig. 3). Trypsin treatment decreases its apparent MW by 3,000 and the released peptide should correspond to residues 101(102)–131 of erythrocyte glycophorin A or tryptic peptide T<sub>4</sub> (ref. 19) located at the COOH-terminal end. When sealed, everted erythrocyte ghosts are treated with trypsin no reduction in the apparent MW of glycophorin A is observed<sup>30</sup>. One possibility is that proteolytic cleavage does occur, but the 3,000 MW difference is not seen because glycophorin A gives a broad and diffuse band on electrophoresis of ghosts. Another, and more likely, possibility is that the COOH-terminal portion of newly synthesised glycophorin A in microsomes is more easily accessible to proteases than the molecule in the finished erythrocyte membrane where it may be covered by other proteins. Treatment of intact cells with trypsin releases much more from the NH<sub>2</sub>-terminal end of the polypeptide in soluble form<sup>15,31</sup>. This indicates that the COOH-terminal part already in the endoplasmic reticulum has a similar location in the red cell membrane.

The biosynthetic pathway of glycophorin A corresponds well to that described for the glycoprotein (G protein) of vesicular stomatitis virus. Glycophorin A has a more complex carbohydrate composition than the viral protein, involving two types of quite different oligosaccharides. The glycosylation of glycophorin A probably involves both lipid intermediates for the synthesis of the *N*-glycosidic oligosaccharide<sup>12</sup> and direct glyco-



**Fig. 4** Our schematic view of the biosynthesis of glycophorin A. GP<sub>a</sub>, glycophorin A precursor; GP<sub>b</sub>, glycophorin A treated with neuraminidase to demonstrate the stage before addition of sialic acid residues; GP<sub>c</sub>, final glycophorin A. ● = hexosamine, ○ = neutral hexose, ▽ = sialic acid. The trypsin cleavage site of GP<sub>a</sub> in microsomes is shown. The asparagine-linked oligosaccharide located at residue 26 in the polypeptide chain is shown in GP<sub>a</sub> in an 'untrimmed' form from the structure described by Li *et al.*<sup>12</sup>.

sylation of the serine/threonine residues from UDP-*N*-acetyl-D-galactosamine<sup>32</sup>. However, similar times are required for insertion into the endoplasmic reticulum membrane, glycosylation and appearance at the cell surface. Whether glycoporphin A contains an additional NH<sub>2</sub>-terminal signal sequence found in most glycoprotein precursors<sup>33</sup> is not known and is being investigated.

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# The three-dimensional structure of tubulin protofilaments

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*A three-dimensional image of tubulin protofilaments, reconstructed to 20 Å resolution from electron micrographs of negatively stained zinc-induced sheets, has been used to generate an improved model for microtubule substructure. This model has been used to phase a reconstructed image from X-ray amplitudes, which is compatible with the electron microscope results.*

THE three-dimensional structure of eukaryotic microtubules has been studied by electron microscopy (EM) of individual specimens<sup>1–4</sup> and by X-ray diffraction of oriented gels of microtubules<sup>5–7</sup>. Results from EM suggested an arrangement of monomer subunits on the same helical surface lattice for both neurotubules and flagellar microtubules<sup>2,3</sup>. Early X-ray diffraction patterns seemed to be in disagreement<sup>5</sup>, but this was resolved after further studies<sup>6,7</sup>; the X-ray data now seem to be quite consistent with the geometry determined by EM. However, there still seem to be some differences in the data, especially with respect to the shapes of the subunits, even at very low (40 Å) resolution. Even different EM studies have tended to give somewhat different impressions of the substructure. In images of brain tubulin sheets, which are thought to be the equivalent of opened-out microtubules, each tubulin subunit seemed to be split longitudinally into two separate domains<sup>2,4</sup>. This feature was not detected in a three-dimensional analysis of intact flagellar microtubules<sup>3</sup>, in which the monomers apparently consisted of single globular units, although the overall outline of the subunits was similar. Furthermore, a model based on an interpretation of the intensity distribution in X-ray diffraction patterns of brain microtubules<sup>7</sup> showed

subunit shapes which were incompatible with either of the classes of image obtained by EM. We now present a new model of the structure of tubulin, based on three-dimensional EM image reconstruction of extended brain tubulin sheets, which seems to explain the differences between the earlier images and suggests a compatible interpretation of the X-ray diffraction patterns from microtubules.

## Zinc-induced tubulin sheets

The specimens we studied were the large two-dimensional crystalline arrays of brain tubulin which assemble in the presence of zinc ions<sup>8,9</sup>. These sheets are apparently made up of the same linear arrays of subunits, or protofilaments, as 'normal' sheets, which are often found in samples of repolymerised microtubule protein and which have been proposed as precursors in microtubule assembly<sup>2,10</sup>. Whereas normal sheets are usually no more than 12 protofilaments wide, presumably because a sheet of 13 protofilaments closes to form a microtubule, zinc-induced sheets may be 100 or more protofilaments wide and are therefore much better for periodic structural analysis. Studies of their projected structure have shown that protofilaments are arranged with alternating polarity (see Fig. 1a), so that these arrays have P2<sub>1</sub> crystallographic symmetry<sup>11,12</sup>. The arrangement is thus quite different from that in a microtubule or a normal sheet, where all the protofilaments have the same polarity. However, the individual protofilaments seem to be very similar, having the same 48–49 Å side-to-side spacing in both types of sheet. This common feature suggests that the rotational orientation of a protofilament about its axis is similar in both structures. By comparing the obliquity of the projected subunit shapes in Fig. 1a with those in computer-filtered EM images of normal tubulin sheets<sup>2,4</sup>, we were also able to deduce that the protofilament images labelled I in Fig. 1 represent the view from the outside of a normal microtubule<sup>11</sup>.

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Figure 1a was derived from four highly selected low-dose EM images of negatively stained specimens and includes data to 16 Å resolution<sup>11</sup>. To obtain a three-dimensional image, this untitled projection was combined with 40 independent images of sheets tilted through angles of 21°, 35° or 51° about various axes<sup>14</sup>. The number of different orientations of the sheets was more than sufficient to provide homogeneous data to 20 Å resolution within the angular range covered by tilting up to 51°. The intensities of most of the reflections fell to noise level within this range. Additional data, corresponding to higher tilt angles, which could not easily be reached by tilting in the microscope, were obtained from images of negatively stained specimens embedded in plastic and sectioned normal to the sheets<sup>13</sup>. These data were to about 40 Å resolution; the complete set of data is therefore still not fully isotropic, so that density variations in the direction normal to the sheets are not quite as well resolved as those in the plane of the sheets. Full details of data collection and analysis are presented elsewhere<sup>13</sup>.

### Three-dimensional model of the sheets

The main features of the reconstructed three-dimensional density distribution are illustrated in Figs 1b and 2. Viewed down its long axis, each protofilament is roughly triangular in shape, with one corner of the triangle protruding from one side of the sheet. Thus, the two exposed surfaces of a protofilament are very different. In longitudinal view, one surface has the form of a longitudinal ridge and the other has a flat ladder-like appearance, with sloping bi-lobed rungs. If we are correct in assuming that the protofilament images labelled I in Fig. 1a represent the view from outside a microtubule, then the ridged surface must be on the outside, while the ladder-like appearance should occur on the inside surface. This is discussed in detail later.

The apparent thickness of the negatively stained sheets, as observed directly in section and also as estimated from the tilted views, is only about 45–50 Å, compared with a difference of 70–80 Å between the inner and outer radii of a microtubule, as estimated from X-ray diffraction measurements<sup>5–7</sup>. Therefore, the sheets have probably shrunk in this dimension as they dried down on to the EM support grid. It is also possible that part of the apparent reduction in thickness results from the masking of

fine surface features extending into the negative stain, or by positive staining of protein on the protofilament surface. Thus, the ridge in particular may extend out further than it seems to in the reconstructed image. The importance of such effects is discussed later.

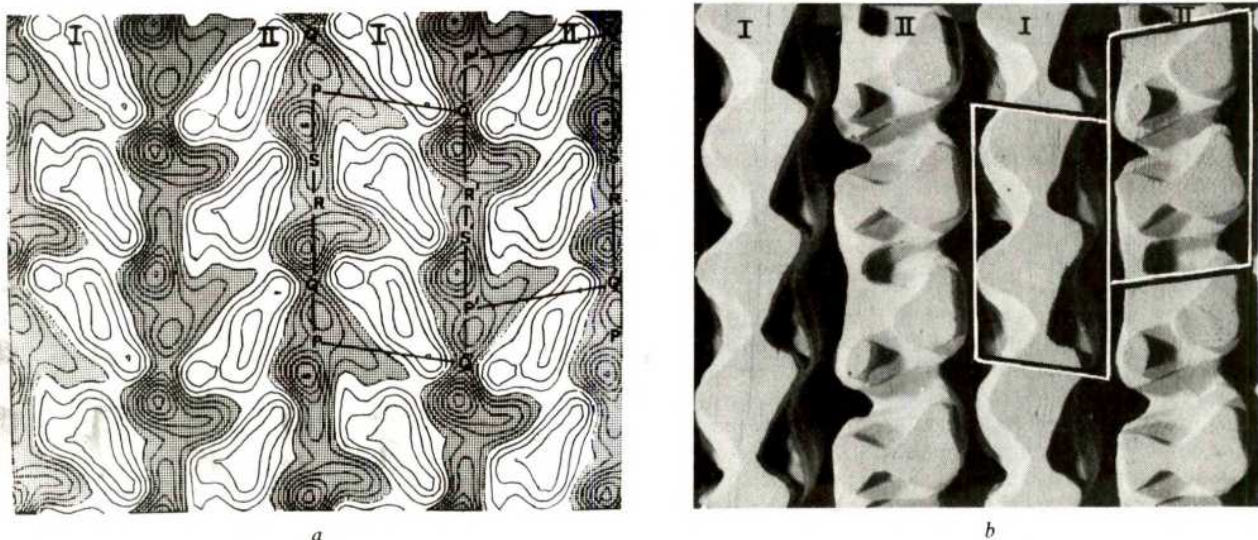
As described previously<sup>11</sup>, each protofilament clearly consists of an alternating linear array of two kinds of slightly different subunits, believed to represent the two species of 55,000 molecular weight monomer which form the tubulin heterodimer<sup>15</sup>. Both kinds of subunit appear in projection as elongated shapes (Fig. 1a), with their long axes at about 40° to the protofilament axes. If one attempts to follow the density corresponding to an individual subunit through successive sections parallel to the central plane of the sheet (Fig. 2), the peaks move gradually downwards between the first and last sections. The subunits thus apparently have the general form of tilted ellipsoids when viewed from the side, as well as face on.

The difference between the two kinds of subunit seems to be rather less in three dimensions than in the two-dimensional projection, showing significantly only at the ladder-like inside surface of the protofilaments. The apparently more definite division into two domains of one of the two subunits presumably represents a real difference in the internal structure of the two monomers.

The pattern of interprotofilament contacts is also not as complex as suggested by the original projection (Fig. 1a), where the contacts between each pair of subunits seem to be represented by two separate regions of higher density in the interprotofilament gap<sup>11</sup>. In the three-dimensional model, the contacts seem to take place over single fairly broad zones (Fig. 2, P to Q or R to S), centred on the central plane of the sheets. The complexity in projection is due to superposition of the intricate stain distributions at the two surfaces of the sheets.

### Model protofilaments rearranged to represent a microtubule

Normal microtubules observed *in situ* consist, in most cells, of 13 longitudinal protofilaments<sup>16</sup> in a polar arrangement<sup>3</sup>. Reassembled microtubules often contain 14 protofilaments<sup>17</sup>, but these are probably aberrant structures. To simulate a normal



**Fig. 1** *a*, Computer reconstructed image of negatively stained zinc-tubulin sheets seen in projection<sup>11</sup>. The variation in density is represented by evenly spaced contour levels. Regions dominated by negative stain are shown shaded while absence of stain, corresponding to protein, is white. Neighbouring longitudinal protofilaments, each 48 Å wide, are related by P<sub>2</sub> symmetry, about screw dyad axes normal to the protofilaments and in the plane of the sheet. Each globular unit along a protofilament is thought to represent a monomer of  $\alpha$ - or  $\beta$ -tubulin. The boxes drawn on the image each enclose what seems to be an 80 Å long  $\alpha$ - $\beta$  heterodimer. *b*, A solid model of part of a zinc-tubulin sheet, with boundaries which follow one of the contour levels in Fig. 2. Two different surfaces of the protofilaments are presented, as adjacent protofilaments face different ways.



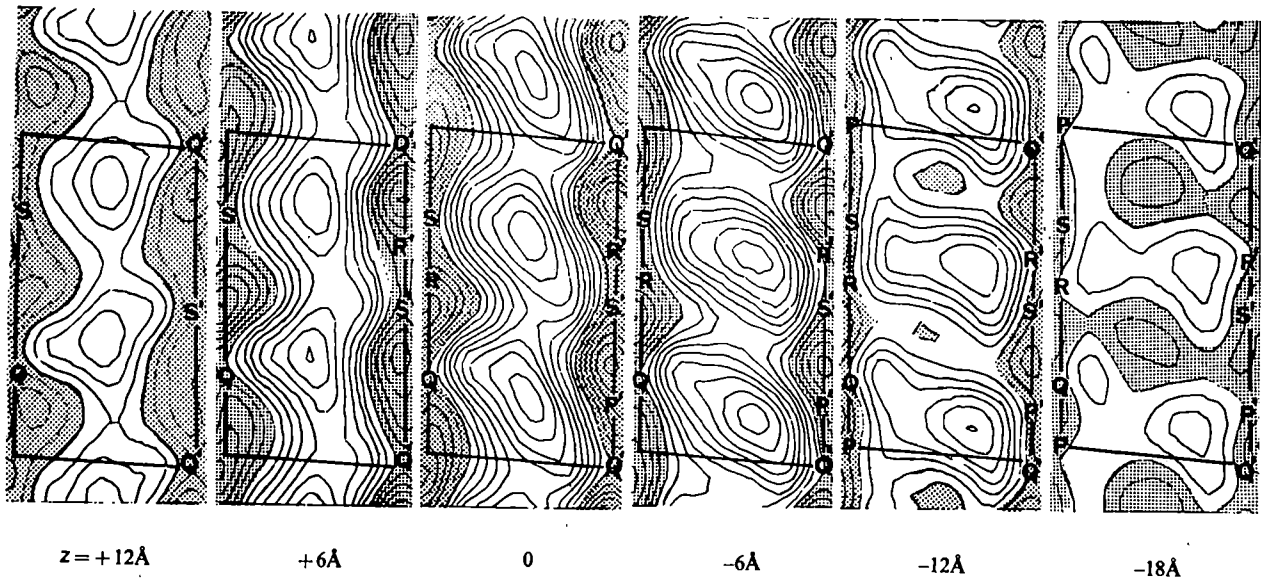
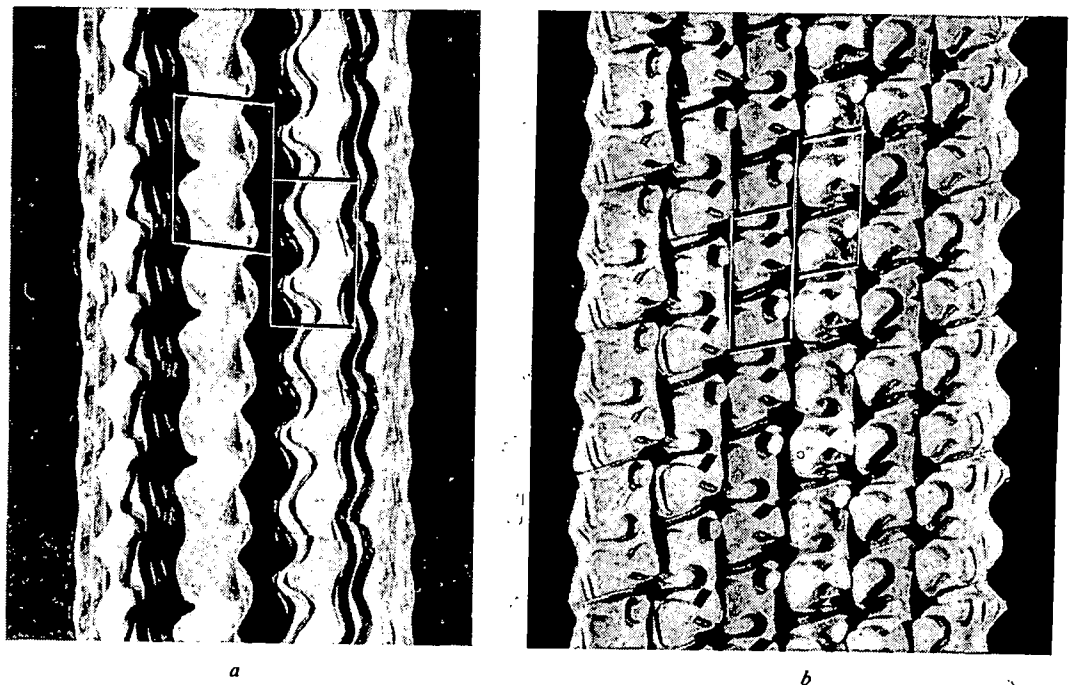


Fig. 2 Sections at different levels ( $z$ ) through one protofilament of the reconstructed three-dimensional model of the negatively stained zinc-tubulin sheets. Below each section is given its distance from the plane through the centre of the sheet. Features at the boundaries of the protofilament are labelled as in the projection in Fig. 1a.

microtubule, therefore, we have arranged 13 model zinc-tubulin protofilaments, as shown in Fig. 3. In this arrangement, the former regions of contact between subunits in the sheet seem to match up again in the tubule, although in different polar orientations<sup>11</sup>. Probably the same hydrophobic regions of each subunit are involved in the assembly of both types of structure. The model in Fig. 3 was assembled with the ridged sides of the protofilaments outermost, so that the projected subunit shapes would agree with images of negatively stained normal sheets<sup>2,4</sup>. There are several observations which support this choice of orientation. One is the good agreement between the appearance of this model and that of the three-dimensional model reconstructed from images of intact flagellar microtubules<sup>3</sup>. In particular, the deep interprotofilament grooves are features of the

outer surfaces of both models. The presence of these grooves at high radius is also strongly indicated by X-ray diffraction data<sup>5-7</sup>. If the wedge-shaped protofilaments were oriented with the ridged surface inwards, grooves would not be formed specifically at the outer surface; the interprotofilament contrast would be weaker and roughly equal at all radii between 70 and 150 Å. Clear images of sectioned normal microtubules<sup>17,18</sup> also support the conclusion that the protofilaments should appear in cross-section as outwardly pointing wedge shapes. A final observation which indicates that, in a normal microtubule, interprotofilament contact is limited to inner radii (less than 110 Å) is that the spacing of protofilaments in flat sheets (normal or zinc-induced) is consistently less (48–49 Å) than the spacing of 53 Å at the mean radius (110 Å) of a microtubule.

Fig. 3 a, Models of zinc-tubulin protofilaments from the reconstructed image shown in Fig. 1b, rearranged to conform to the helical symmetry established for a normal microtubule. Thus, the monomer subunits lie on a family of three shallow left-handed helices. The relative arrangement of tubulin dimers is that observed in complete flagellar microtubules<sup>3</sup> (see text). Two unit cells, each enclosing an 80-Å long  $\alpha$ - $\beta$  dimer, are outlined. b, Inside surface of part of a microtubule, opened out to form a 'normal' sheet. The polar arrangement of the protofilaments is the same as in a. Comparison of these images with the



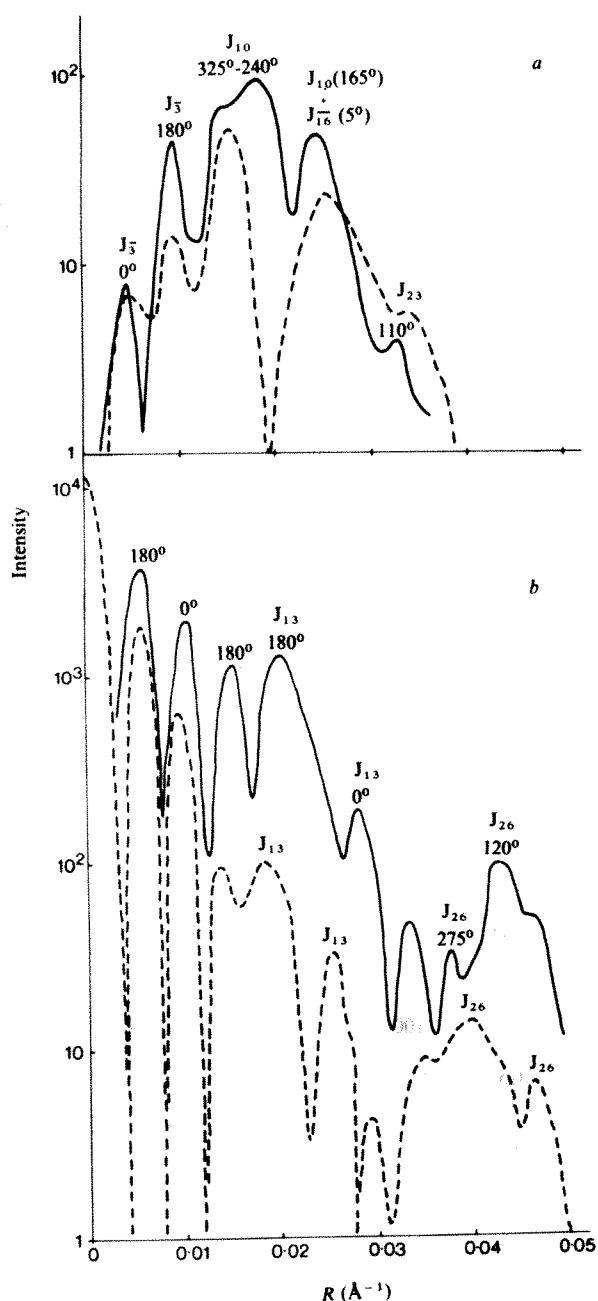
reconstructed three-dimensional model of intact flagellar microtubules<sup>3</sup>, suggests that the bottom of the model, as shown in these two views, corresponds to the basal end of a flagellar microtubule.

The other assumption incorporated in the model shown in Fig. 3 is that the subunit dimers in adjacent protofilaments are in a staggered arrangement, as observed in complete flagellar microtubules, that is, the A-tubules of outer doublet microtubules<sup>3</sup> and the central singlet microtubules<sup>1</sup>. This is in fact the only helically symmetrical arrangement possible for a 13-protofilament microtubule in which the monomer subunits are arranged as shown. There is some evidence<sup>7,12</sup> that brain tubulin may sometimes reassemble *in vitro* in an alternative arrangement similar to that in a flagellar B-tubule<sup>3</sup>, but when microtubules are reassembled from brain extracts containing a full complement of so-called microtubule associated proteins, the latter, seen as projections from the outsides of the tubules, seem to follow the symmetry of the A-tubule lattice<sup>19</sup>. This point is discussed in more detail elsewhere<sup>20</sup>.

### Variations in EM images

It is now understandable why somewhat variable images have been obtained in previous EM studies of protofilament structure. The appearance seems to vary according to which of the two different protofilament surfaces is the more strongly

emphasised by the negative stain. The bi-lobed, or ladder-like appearance obtained for opened-out brain microtubules<sup>2,3</sup> corresponds rather closely to the appearance of the inner surface of the present model. Erickson<sup>2</sup> noted that the normal sheets, being curved in solution, always fell with the outer surface next to the carbon substrate of the EM grid. Presumably the inside surface, which was thereby most exposed to the negative stain subsequently applied to the grid, was more strongly contrasted than the outer surface. The outer surface of the three-dimensional image reconstructed from images of intact flagellar microtubules<sup>3</sup>, on the other hand, closely resembles the outer surfaces of the protofilaments in the present model, whereas the inner surface showed much less detail. In the case of an intact microtubule, it seems that the outside is most strongly contrasted by negative stain. In the zinc-induced sheets the orientation of the protofilaments alternates. Differential staining of the two sides of such sheets probably explains the great difference in projection of adjacent protofilaments observed initially by Crepeau *et al.*<sup>4</sup>. However, the images we have used all show very good P2, symmetry<sup>11</sup>, so we deduce that in our specimens each protofilament must be more or less equally well contrasted on both sides. The three-dimensional image reconstructed from



**Fig. 4** Intensity distributions for the equatorial (b) and 40 Å (a) layer lines of diffraction patterns from real and model microtubules. The dashed lines represent the intensity distributions measured by Mandelkow *et al.*<sup>7</sup> in X-ray diffraction patterns obtained from oriented hydrated gels of microtubules. The solid lines follow cylindrically averaged intensity distributions calculated for a model microtubule, consisting of 13 zinc-tubulin protofilament images arranged on a mean radius of 110 Å; the density distributions were uniformly 'stretched' by 30% in the direction normal to the zinc-tubulin sheets, to correspond more closely to the microtubule dimensions estimated from X-ray diffraction data<sup>7</sup> (see text). The only significant effect of this is a slight change in the radial positions of the intensity peaks in the calculated diffraction pattern. The relative phases (for protein represented as positive density) determined for the various helical contributions are indicated above the intensity peaks. On the calculated equator, the strong peaks near the origin represent the transform of a hollow cylinder. The peaks at 0.020 Å<sup>-1</sup> and 0.028 Å<sup>-1</sup> (labelled J<sub>13</sub>) arise mainly from the division of the cylinder into 13 distinct longitudinal protofilaments: the first is from 13-fold contrast at the outside of the cylinder, the second from the much weaker contrast on the inside. Second order diffraction from the protofilaments provides the main contributions to the last group of peaks (J<sub>26</sub>): the weak inner peak of this group corresponds to features of the outside of the cylinder, the stronger peak to the inner side. On the 40 Å or second layer line (based on an axial repeat of 80 Å) of the calculated diffraction pattern, the two (J<sub>-3</sub>) peaks close to the meridian arise from outer and inner radii contributions from the family of three shallow left-handed helices; the apparently double third peak (J<sub>10</sub>) (with varying phase across it) consists mainly of contributions from the 10 steeper, right handed helices at outer radii. The broad fourth peak includes a small contribution from the latter family at inner radius, but also significant contributions from the left-handed 16-member family at radii centred on a mean of about 110 Å. The calculated curves do not correspond exactly with those measured from X-ray diffraction patterns; for example, the relative intensities on the equator fall off differently and the J<sub>10</sub> peak on the 40 Å layer line has a different shape. However, some differences are to be expected, as the model was derived by studying dehydrated protein embedded in metal stain, whereas the X-ray diffraction pattern is from hydrated unstained specimens and provides information about the contrast between protein and aqueous solvent.



them should therefore provide a more reliable impression of the structure than has been obtained from previous EM studies of tubulin structure.

However, because of artefacts due to dehydration, radiation damage and the limitations of negative stain contrast, the results obtained by EM are not wholly satisfactory for studying the native structure of proteins. Low-dose electron microscopy of unstained specimens, embedded in glucose or sucrose solutions to alleviate the effects of dehydration, has proved successful in studies of the substructure of certain thin two-dimensional crystals of biological origin<sup>14,21</sup>. However, the results obtained so far with tubulin sheets<sup>4</sup> have not been very promising, the resolution attained being somewhat less than that obtained from negatively stained specimens. The zinc-tubulin sheets, although they grow much larger than normal tubulin sheets, probably still do not allow sufficient averaging to compensate for the extremely low signal-to-noise ratio in EM images of unstained protein.

### Diffraction patterns from microtubules

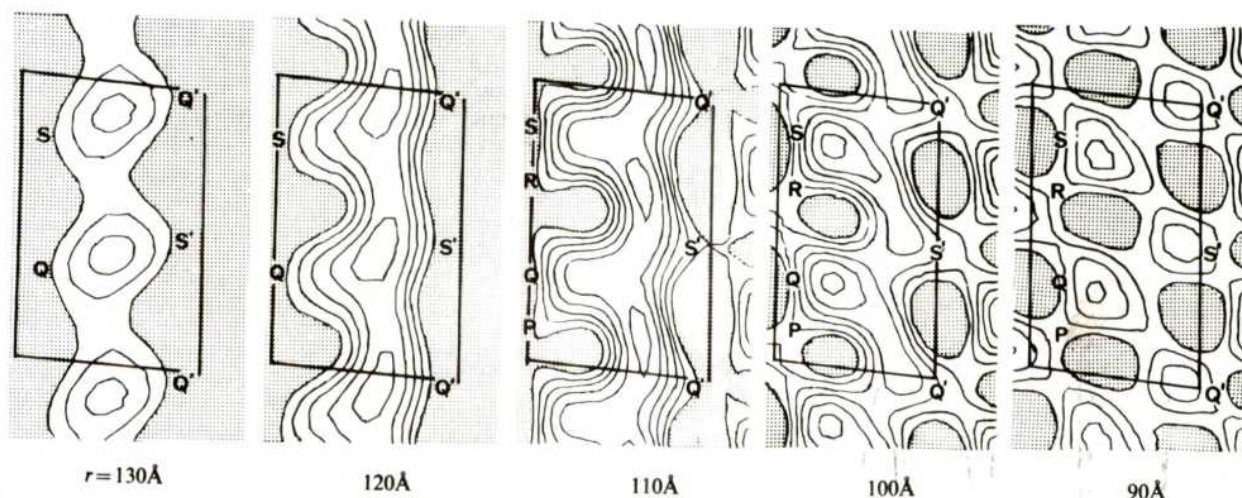
X-ray diffraction of oriented gels of hydrated microtubules provides information about the protein in its native state, but the X-ray patterns from native specimens do not provide sufficient data by themselves for the reconstruction of a three-dimensional image of the structure. One way of trying to supply the missing information at low resolution is to use phase information obtained by electron microscopy<sup>7</sup>. For this purpose, we have calculated a three-dimensional diffraction pattern for the model density distribution shown in Fig. 3. The calculated diffracted intensity distributions, averaged around the vertical axis to simulate diffraction from an oriented gel of microtubules, are shown in Fig. 4 for the equatorial and 40 Å layer lines, together with the corresponding relative intensities measured from X-ray diffraction patterns. These curves calculated for the rearranged zinc-tubulin protofilaments are surprisingly more similar to the X-ray curves than are the distributions obtained directly from optical diffraction patterns of negatively stained microtubules<sup>1-4,7</sup>. In the latter case, the peak closest to the meridian on the 40 Å layer line is usually the strongest on that

layer line, a peak corresponding to the third peak on the X-ray 40 Å layer line is much weaker in the microtubule optical diffraction patterns and the other two X-ray peaks are absent. As discussed above, this seems to be because negative stain does not map out the surface of a protofilament in an intact microtubule as closely as it does that of a protofilament in a zinc-tubulin sheet.

### Interpreting the X-ray patterns

Cohen *et al.*<sup>6,7</sup> have interpreted the low angle X-ray intensity distribution (Fig. 4, dashed lines) in terms of diffraction mainly from the inner and outer surfaces of the microtubules, which apparently have inner and outer radii of 70 and 150 Å respectively. The strong equatorial  $J_{13}$  and  $J_{26}$  reflections have both been identified as coming from the outer surface, with mean radii of 126 and 115 Å respectively. The series of four peaks on the 40 Å layer line were indexed as two  $J_{-3}$  contributions from outer and inner mean radii of 130 and 70 Å, followed by two  $J_{10}$  contributions from outer and inner mean radii of 120 and 70 Å.

Mandelkow *et al.*<sup>7</sup> attempted to reconstruct a three-dimensional model of a microtubule from the measured X-ray intensities, interpreted in the above manner, by combining them with phases determined by Erickson<sup>2</sup>, and confirmed by their own observations, from two-dimensional EM images of microtubules opened out as sheets. (In calculated diffraction patterns from digitised images, with a phase origin equivalent to that used in Fig. 4, phases of 180° are found for each of the lattice points corresponding to single  $J_{13}$ ,  $J_{26}$ ,  $J_{-3}$  and  $J_{10}$  contributions.) However, as the relative intensities in the X-ray diffraction pattern and the diffraction patterns from the original EM images were very different, the hybrid reconstructed image<sup>7</sup> was also quite different from the original images, with elongated subunits sloping in the opposite direction. Without reasonable agreement between the two techniques, there is no justification for combining EM phases with X-ray amplitudes. It is now possible, however, from our analysis of the zinc-tubulin structure, to suggest some modifications to the above interpretation and phasing of the X-ray patterns, which lead to a much closer consistency between the X-ray and EM data.



**Fig. 5** Sections at different radii ( $r$ ) through part of the reconstructed density distribution, calculated using Fourier amplitudes deduced from X-ray diffraction patterns of intact brain microtubules and phases (see Fig. 4) derived from model zinc-tubulin protofilaments rearranged as shown in Fig. 3. Features of the protofilament outline, which seem to correspond to those in the sections through the original image (Fig. 2), are labelled P to S. The two images are rather different at smaller radii; the sloping regions of high density (S to S') and (Q to Q') have a steeper slope than the corresponding regions in Fig. 2. (This difference seems to be less for one type of subunit in Fig. 2 than for the other.) As well as the equatorial and second (40 Å) layer lines shown in Fig. 4, the data included weak contributions from the third and fourth layer lines, estimated from the published X-ray patterns<sup>7</sup>. (Note that the indexing of the odd layer lines is determined by the arrangement of 80 Å long tubulin dimers, which is discussed below Fig. 3.) On the third layer line, an apparent  $J_2$  contribution to the X-ray diffraction patterns was included with a phase of 45° but being weak has very little effect. The fourth layer line data consisted of approximately equal  $J_{-6}$  and  $J_7$  contributions with phases of 210° and 50°, based on the model calculations.



The main difference from the previous interpretation is the source of the fourth peak from the meridian on the 40 Å layer line. Calculations from our model suggest it is probably a mixture of different contributions. As well as a small  $J_{10}$  contribution, it is likely to include a major  $J_{-16}$  contribution, with a mean radius of about 110 Å. It is not completely clear why a  $J_{-16}$  contribution is never observed in optical diffraction patterns from negatively stained microtubules or normal tubulin sheets, but it is probably for the same reason that the  $J_{10}$  contribution is weaker than the  $J_{-3}$  contribution, instead of stronger as in the X-ray patterns. The  $J_{-3}$  reflections clearly do come from the innermost and outermost surfaces of the structure and must be relatively easily revealed by negative stain, provided the surface in question is exposed to it. On the other hand, according to the present interpretation of the X-ray data, both the major  $J_{10}$  and  $J_{-16}$  contributions come from radii near the middle of the protein layer, into which it is presumably more difficult for the stain to penetrate. It may be relevant in this context that the X-ray diffraction patterns from dehydrated microtubules are said to be more similar to the optical diffraction patterns from electron micrographs<sup>5,6</sup>. This suggests that dehydration may cause the cleft between protofilaments to close up at inner radii and prevent features at these radii from contributing to the diffraction patterns.

### Phasing the X-ray data

The relative phases we assign to the various contributions (Fig. 4), which were derived from the model shown in Fig. 3, also differ from those used by Mandelkow *et al.*<sup>7</sup>. This is mainly because, in the images of the flat sheets which supplied their phases, contributions from different radii in the microtubules become superimposed in projection. Unless features at all radii have corresponding spatial frequencies exactly in phase, the relative phases in the transform of such a flattened sheet will not agree with a separated set of either inside or outside surface contributions.

One significant difference is in the values assigned to the equatorial  $J_{26}$  intensity peaks. In EM images, the feature giving rise to the strongest diffraction contribution of this order is the shallow longitudinal groove bisecting one surface of each protofilament. We interpret this surface as being on the inside of a microtubule. In the X-ray diffraction patterns, the strongest  $J_{26}$  contribution seems to come from a radius of about 115 Å, which is within the extent of the deep interprotofilament grooves on the outside surface of the microtubule. Mandelkow *et al.*<sup>7</sup> originally related this contribution to the apparent splitting of the protofilaments (hence the 180° relative phase they assigned to it), but we now interpret it as most likely due to the sharpness of the ridges on the outer surfaces of the protofilaments. A smaller peak further out on the equator, at  $R = 0.047 \text{ Å}^{-1}$ , probably arises from the apparent splitting of the protofilaments at inner radii.

According to this interpretation, the relative intensities of the two  $J_{26}$  contributions, from the inner and outer surfaces of the microtubules, are reversed in the X-ray diffraction patterns and the patterns calculated from the rearranged zinc-tubulin model. This could be due to artefacts of negative staining whereby the tips of the ridges on the outer surface of each protofilament might be swamped by negative stain and the outer surface  $J_{26}$  contribution thus reduced, while the shallow groove down the centre of the inside surface of the protofilament would tend to be over-emphasised by the stain, causing the inner surface  $J_{26}$  contribution to be enhanced.

### Model from combined X-ray and EM data

A test of the above interpretation of the X-ray patterns is to see whether a reconstructed image, based on the X-ray amplitudes and the phases derived from the rearranged model, resembles the results from EM alone. Figure 5 shows sections through one protofilament of such a calculated microtubule density distribution. This structure can be compared with the zinc-tubulin

image shown in Fig. 2, bearing in mind that the latter structure seems to have shrunk by 30–40% in the  $z$ -direction. There are some other differences between the two structures, as expected from the differences in the two sets of diffraction curves in Fig. 4. The sections in the region of the outside surface (around  $z = 12$ , 6 Å in Fig. 2; around  $r = 130$ , 120 Å in Fig. 5) are reasonably similar, but at inner radii there are some distinct differences. In particular, the 'rungs of the ladder' (around  $z = -18$ , -12 Å in Fig. 2; around  $r = 90$ , 100 Å in Fig. 5) seem much more steeply tilted, because of the greater strength of the  $J_{-16}$  contribution to the data, relative to the  $J_{-3}$  contribution. Also, the differences between the two species of tubulin subunit are much less obvious, because of the relative weakness of the third (27 Å) layer line in the X-ray diffraction pattern (no data from other odd-numbered layer lines were included).

We do not know the reason for these remaining differences. Discrepancies in the central sections of the protofilaments could obviously be due to failure of the negative stain to penetrate fully into this region, even in the case of the zinc-tubulin sheets, which seem to stain better than normal tubulin structures. But this explanation is not adequate for the differences observed in the surface structure. One must postulate that the negative stain does not adequately represent the native protein contours. This might be due to selective positive staining<sup>22</sup> of certain parts of the protein molecules. Alternatively, there may be reproducible changes in conformation during negative staining and dehydration of the EM specimens, as there seems to have been a consistent shrinkage of the sheets. Neither of these possible effects could be corrected for when the negatively stained density distribution was 'stretched' (Fig. 4) to adjust the dimensions to those of hydrated protein. Because of this lack of exact correspondence between the two images, we still cannot be sure that the relative phases, especially those related to features on the inside of the cylinder, are quite correct.

Resolution of the remaining uncertainties in the structure at this resolution must await an independent determination of the Bessel function assignments and phases in the X-ray diffraction pattern, for example, by labelling the protein with heavy atoms<sup>23</sup>, if this proves possible. However, the consistency between the results obtained by electron microscopy and X-ray diffraction is clearly much better than it originally seemed to be<sup>5-7</sup> and, for this reason, we believe that the three-dimensional model of zinc-tubulin protofilaments presented here is a good first approximation to the low resolution structure of assembled tubulin.

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# letters

## Polar clearing in the Venus clouds observed from the Pioneer Orbiter

PIONEER Venus 1 was put into a 24-h orbit around the planet on 4 December 1978. Since then, it has made remote sensing observations of the clouds and the overlying atmosphere at IR, visible and UV wavelengths. The IR instrument includes a channel at a wavelength of  $11.5\ \mu\text{m}$ , which is used to measure the effective temperature of the cloud tops. Carbon dioxide, and all of the known constituents of the gaseous atmosphere, are highly transparent at this wavelength. Earth-based telescopic observations at similar wavelengths<sup>1-3</sup> have generally shown fairly uniform temperatures of the order of 240 K across the face of Venus. The accepted interpretation has been that the cloud cover on Venus not only covers the whole planet, but is also extremely uniform. The early Pioneer observations confirmed this general picture, for the equatorial and mid-latitudes which comprise most of the surface area of the planet<sup>4</sup>. Interesting and meteorologically important structure with diurnal, seasonal and random components is found in the Pioneer measurements, as it was in earlier ground-based and spacecraft observations, but this structure has an amplitude of 10 K or less<sup>1-6</sup>. However, these subtle contrasts which dominate thermal maps of lower latitudes change to dramatic structure near the pole. Even the Earth-based observers, who must view the polar regions on Venus at very oblique angles, have reported cold bands surrounding one or other of the poles at various times<sup>2</sup> and occasional polar 'hot spots'<sup>1</sup>. The Pioneer orbit passes almost directly over the pole (inclination =  $105^\circ$ ) and so provides the first good views of this interesting region. The IR instrument was

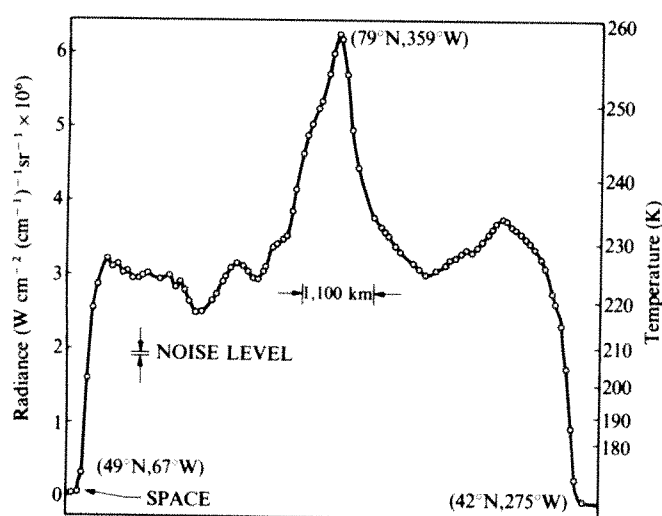
operated only in its low spatial resolution mode for the first part of the mission. Results from those observations<sup>4</sup> showed a wave-shaped collar of high, cold cloud surrounding the north pole, with warmer cloud temperatures polewards. Close to the pole itself, the observed temperature is the highest anywhere on the planet, not only at the cloud top but also in the overlying atmosphere<sup>4</sup>. We report here the first high data rate, high spatial resolution observations of the polar region. These were obtained on the tenth orbit of Venus on 15 December 1978.

Figure 1 shows a single swath across the planet, covering latitudes from about  $40^\circ$  to  $80^\circ\text{N}$  as shown in Fig. 2. Figure 3 shows an image of the region from a series of contiguous swaths. The individual swaths are obtained as a result of the spinning motion of the spacecraft, which rotates at  $\sim 5$  r.p.m. The separation of the swaths is due to the motion of the spacecraft along its orbit.

These data show an extremely localised, very warm feature at about  $80^\circ\text{N}$  and  $359^\circ\text{E}$  (corresponding to a local time of day of about 9 am). The peak temperature of 260 K is the highest cloud-top temperature ever to be observed on Venus. Because of the abrupt nature of the increase, and also because the Pioneer IR radiometer makes independent measurements of the temperature of the clear atmosphere above the clouds, it is evident that the large temperature contrast is due to a depression or clearing of the cloud layer rather than to an actual temperature increase at a constant height level. This in turn is probably due to strong downwelling in the atmosphere near the pole. To account for the contrast of approximately 45 K between the peak of the anomaly and the nearby cold band which surrounds it, a difference of cloud height of approximately 15 km is required. This follows because the measured temperature lapse rate in the polar region is close to  $3\ \text{K km}^{-1}$  (ref. 5). Cloud height differentials of this magnitude are seldom, if ever, observed on the Earth.

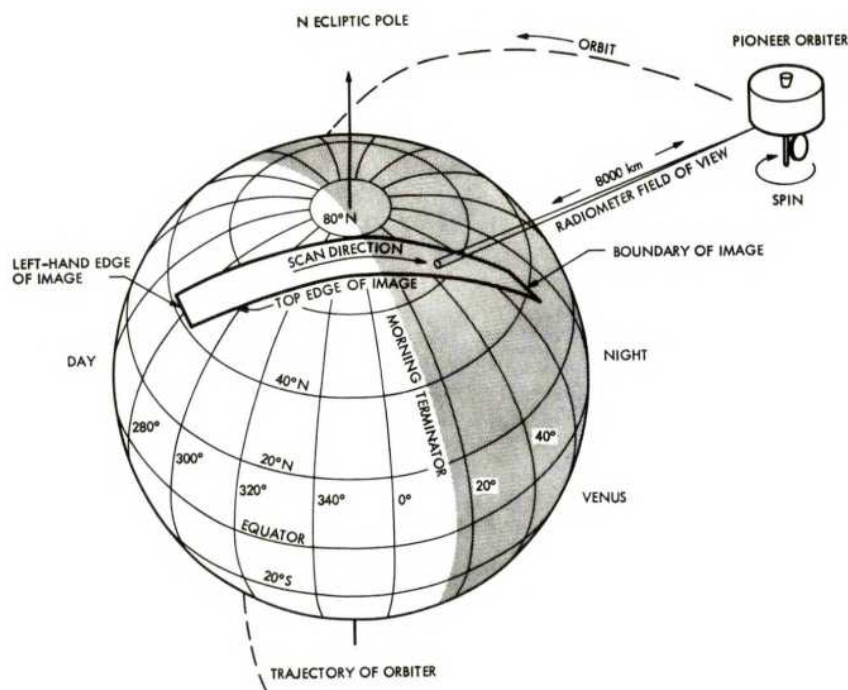
It is interesting to consider how high the temperature would have to rise in an anomaly of this kind in order to correspond to a completely cloud-free atmosphere. To investigate this, we have calculated the transmission profile of a model Venus atmosphere corresponding to the spectral bandpass of the  $11.5\ \mu\text{m}$  channel. As stated previously, this wavelength was selected because it is one of the most transparent in the thermal IR. However, because of the high concentration of  $\text{CO}_2$  on Venus, even this would become opaque (defined as an optical depth greater than unity) by a depth corresponding to a pressure of about 0.8 bar. At this level, the atmospheric temperature is approximately 290 K. Thus, the highest temperature measured approaches, but definitely does not attain, that which would correspond to a totally cloud-free region. However, as can be seen from Fig. 3, the high resolution data sequence ended before the coverage of the feature was completed. The temperature may have risen higher, perhaps even to the temperature corresponding to a total absence of cloud, nearer to the pole itself. An analysis of further high-resolution scans obtained later in the mission will eventually test this hypothesis and also reveal how the feature evolves.

It can be affirmed that the observations presented above constitute new and persuasive evidence for the presence of strong atmospheric subsidence near the poles. This fits very well with the suggestion by Murray *et al.*<sup>7</sup> and Suomi and Limaye<sup>8</sup> of vortex-type circulation at the poles in the UV imaging sequences of Venus by Mariner 10. Suomi and Limaye<sup>8</sup> state that these



**Fig. 1** A single scan across Venus at a wavelength of  $11.5\ \mu\text{m}$  (spectral resolution =  $0.027\ \mu\text{m}$ ) showing the effective cloud-top temperature observed. The scan cuts through the cold polar collar, showing its asymmetry, and through the anomalously hot feature near to, but offset from, the north pole. The scan corresponds to the bottom edge of the image in Fig. 3.





**Fig. 2** Diagram showing the geometry of the observations, and the boundaries of the high-resolution image.

observations of spiral bands of cloud and a poleward component of cloud motion "are compelling suggestions that at least during the 7 days of the Mariner 10 flyby in 1974 the stratospheric circulation was composed of two giant vortices more or less centred on each pole with meridional inflow from low latitudes towards each pole. The vortex . . . would be characterised by a region of mass sink in the polar regions in the upper atmosphere and a mass source at lower latitudes, essentially a hemispheric Hadley circulation cell strongly organised by the vertical zonal flow".

The 'single cell' model for the Venus stratosphere is attractive, not least because it provides an intuitive explanation for the ubiquitous and uniform cloud cover outside the polar domains. It is easy to imagine that the atmosphere may be slowly rising over most of the area of the planet, thus sustaining dynamically the observed cloud deck. This mass flow would then be balanced by relatively rapid descent within the polar vortices. There are difficulties with this, however. First, it is difficult to explain why the 'eye' of the vortex is not centred on the rotational pole. The low resolution data alluded to earlier show clearly that its centre is  $5^{\circ}$ – $10^{\circ}$  from the pole itself. Second, the presence of the thick, high polar collar cloud (segments of which can be seen in Fig. 3) is not explained by a simple vortex circulation and may, in fact, preclude it. Third, the downwelling in the polar region must be explained in terms of a circulation mechanism which produces a poleward meridional flow<sup>8</sup>, but against the gradient of increasing temperature from equator to pole<sup>4</sup>. Such a situation is not



**Fig. 3** An 11.5- $\mu\text{m}$  image of thermal emission from the region near the north pole of Venus delineated in Fig. 2. The data were obtained from the Pioneer orbiter on 15 December 1978. The brightest area is emitting more intensely, and hence is warmer, than its surroundings. The dark (cold) circumpolar collar appears either side of the hot region, and some of its detailed structure can be seen. The limb of the planet is visible at either edge of the frame.

consistent with a classical 'Hadley' type circulation driven by solar heating. The stratospheric dynamics on Venus require some more complex mechanism, perhaps involving motions driven from below.

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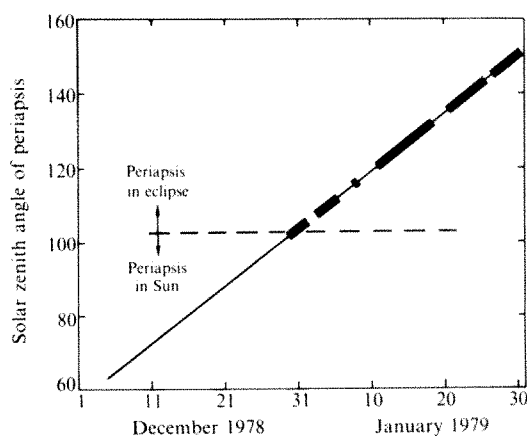
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## Evidence for lightning on Venus

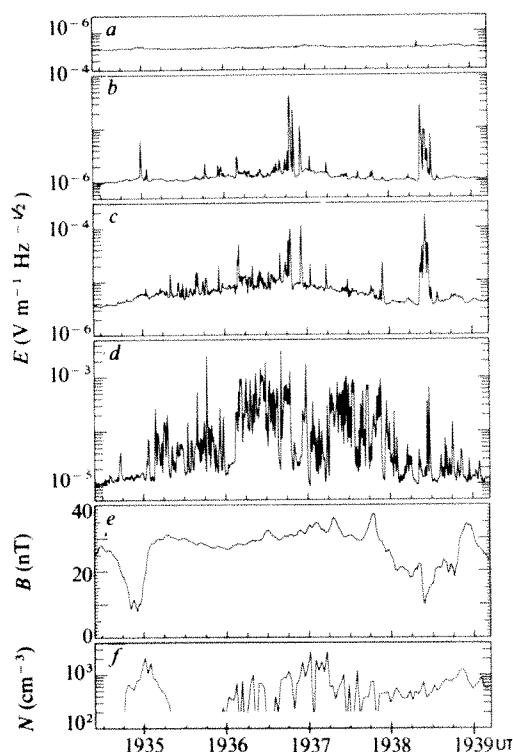
WHETHER lightning exists in a planetary atmosphere is a fundamental question for the atmospheric physicist. The conditions within a lightning stroke are far different from those within ambient atmosphere, permitting chemical and physical changes in the atmosphere which are not possible in equilibrium conditions. The relative importance of such nonequilibrium processes depends on the frequency and the location of this electrical activity. Discovery of lightning on other planets would also affect other scientific fields, including studies of planetary magnetospheres and of life. Lightning has been predicted or considered on Venus<sup>1</sup> and Jupiter<sup>2,3</sup>, and evidence for lightning has been obtained with instrumentation on Venera 11 and 12 descent vehicles<sup>4</sup>. Electric fields characteristic of lightning, and acoustic signals, were detected on the dayside of the planet. We



**Fig. 1** The solar zenith angle of the periastron of the Pioneer Venus Orbiter in December 1978 and January 1979 with orbits with impulsive events marked. The events, possibly due to lightning in the atmosphere of Venus, seem to be observed only when the Orbiter is in the shadow of Venus.

present here evidence of lightning on Venus obtained by instruments on the Pioneer Venus Orbiter.

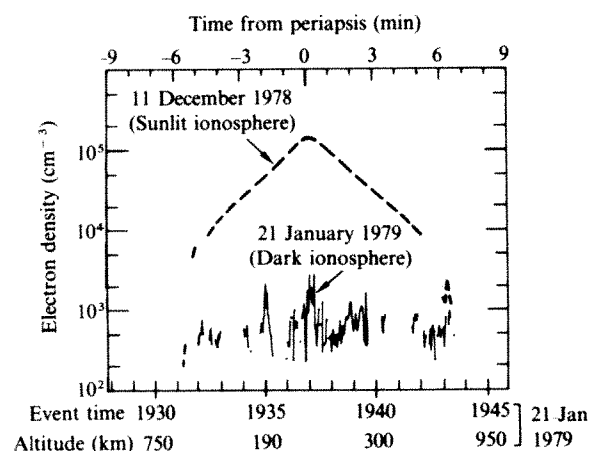
The spacecraft was placed into Venus orbit on 4 December 1978<sup>5</sup>. Results discussed here are derived from observations to 31 January 1979. The periastron of the Orbiter is well within the Venus ionosphere where electromagnetic waves characteristic of lightning might propagate. Initially periastron was in sunlight. However, periastron moved into darkness by the end of December, and at this time the Orbiter electric field detector obtained its first evidence for lightning.



**Fig. 2** A few minutes of electric and magnetic field data observed with Pioneer Venus Orbiter on 21 January, 1979. The measured electric field amplitudes of waves with frequencies *a*, 30 Hz; *b*, 5.4 kHz; *c*, 730 Hz; *d*, 100 Hz. The magnitude of the magnetic field is shown in *e*. Electron density data are shown in *f* (unconnected for densities  $< 500 \text{ cm}^{-3}$ ). Periastron was at 19 h 36 min 32 s.

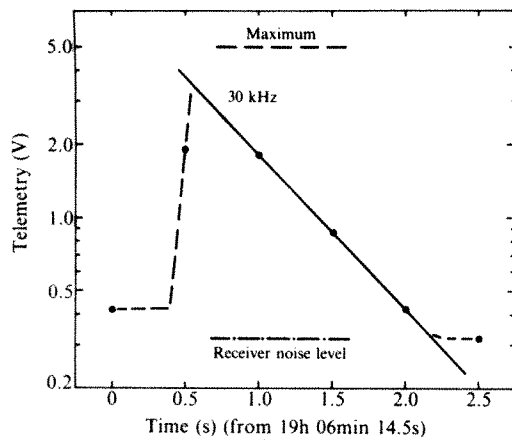
The electric field detector has sensors which are mounted on the body of the Orbiter spacecraft with an effective antenna length of 0.7 m. The amplitude of the voltage induced on the antenna is measured in four frequency bands centred at 0.100, 0.730, 5.40 and 30.0 kHz, each 30% wide. The amplitudes are telemetered at various rates, commonly a complete spectrum (four measurements) every 0.5 s. The data reported here are from three instruments on Pioneer Venus—the electric field detector<sup>6</sup>, the magnetometer<sup>7</sup>, and the Langmuir probe<sup>8</sup>.

Electric field data from early orbits were carefully inspected for evidence of lightning but none was found. In fact, in the dayside ionosphere, the wave levels were particularly low during these early orbits, especially in the two lower bands. Beginning with periastron data on 30 December 1978, strong, very impulsive signals were often observed at low altitudes. Figure 1 shows the precession of the solar zenith angle of the periastron of the orbit with the days of the low altitude impulsive events indicated. Reception of the waves at the satellite appears possible only when the satellite is in or near the shadow of Venus. In principle, this could be due to a source effect (for example, if venusian thunderstorms occur only at night) or to a propagation effect (for example, if the daytime venusian ionosphere is opaque to the waves). A source effect can probably be ruled out since Venera 11 and 12 lightning observations were made at solar angles  $< 25^\circ$ , and this fact alone suggests that the nightside observations on the Orbiter are associated with changes in the ionosphere.



**Fig. 3** Onboard measurements of the electron density for a typical dayside and nightside ionosphere (dashed line) and for the nightside ionosphere corresponding to the data shown in Fig. 2 (solid line).

Figure 2 shows a fairly typical example from 21 January 1979 when the altitude of periastron was 164 km (at 19 h 36 min 32 s). The impulsive wave events were primarily detected at altitudes less than about 250 km, with regions of low electric field activity in the ionosphere above about 250 km. The magnetic field was about 30 nT during most of the time shown in Fig. 2, giving an electron gyrofrequency of about 840 Hz. Within the density 'dropouts' we use  $N \approx 40 \text{ cm}^{-3}$  as an electron density estimate. For a magnetic field of 30 nT and an electron density of  $40 \text{ cm}^{-3}$ , 100 and 730 Hz waves will propagate in the whistler mode, whereas no cold plasma wave mode will support propagation at 5.4 or 30 kHz. The events are strongest at 100 and 730 Hz although a few events extend to 5.4 kHz as well. The 5.4 kHz data may be due to propagation in other wave modes or may be due to waves leaking out of a nearby region of depleted electron density (common on the nightside of Venus). Figure 3 shows the different character of the ionosphere on the day and night sides of Venus. The dayside is relatively more dense and more regular. The impulsive events occurred at an average rate of about  $0.5 \text{ s}^{-1}$  between 19 h 35 min and 19 h 39 min. The actual



**Fig. 4** The decay of the measured amplitude of an impulsive event on 13 January, 1979. The decay is exponential and apparently due entirely to the electronic averaging in the instrument. This indicates that the length of the input signal was very much less than the time constant of 0.70 s.

rate may have been much higher since the time between measurements was 0.5 s during this period. Venera 11 observed a maximum impulse rate of  $25 \text{ s}^{-1}$  (ref. 1).

Lightning on Earth is very impulsive. A test of the impulsiveness of the events is to observe the decay of the receiver output after an isolated impulse. The pulse shape of such an isolated impulse on 13 January 1979 is shown in Fig. 4. The rise time of the pulse is very short but, as expected, the decay is exponential with a time constant of 0.70 s, consistent with the decay time constant of the receiver.

The following evidence leads to the tentative conclusion that the impulsive events were caused by venusian lightning: (1) The signals are intense and highly impulsive; (2) the signals are detected near periapsis, well inside the ionosphere; (3) the spectral characteristics of the signals are generally consistent with whistler wave propagation up through the ionosphere; (4) the signals are often observed during intervals when low and variable electron densities are measured.

When the nightside densities are as high as  $10^3 \text{ cm}^{-3}$ , the interpretation that the impulsive events were caused by lightning requires that the whistler mode damping that seems to be effective in the dayside ionosphere<sup>6</sup> should be ineffective in the lower density nightside ionosphere. Other interpretations of some of the wave measurements are also conceivable (for example, some observations could represent electrostatic ion acoustic or electrostatic ion cyclotron waves<sup>9,10</sup>, or be due to turbulence near zero frequency<sup>11</sup>). However, many of the impulsive events are detected near regions with local electron densities  $< 10^2 \text{ cm}^{-3}$ , and since the electron energy for Landau damping is proportional to the inverse of the density, the cool electrons near periapsis at night can never provide much damping. Moreover, for densities  $< 10^2 \text{ cm}^{-3}$  and a magnetic field of 30 nT, a 100-Hz whistler mode wave has a wavelength of 10 km or more, and it is almost certain that the spacecraft is frequently as close as the wavelength from the lower edge of the ionosphere. For these cases, and others where ducts might be present, signals from atmospheric lightning should be able to propagate to the spacecraft without any significant damping.

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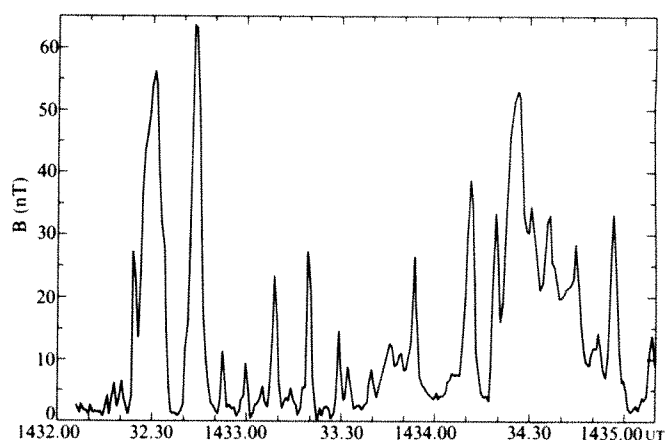
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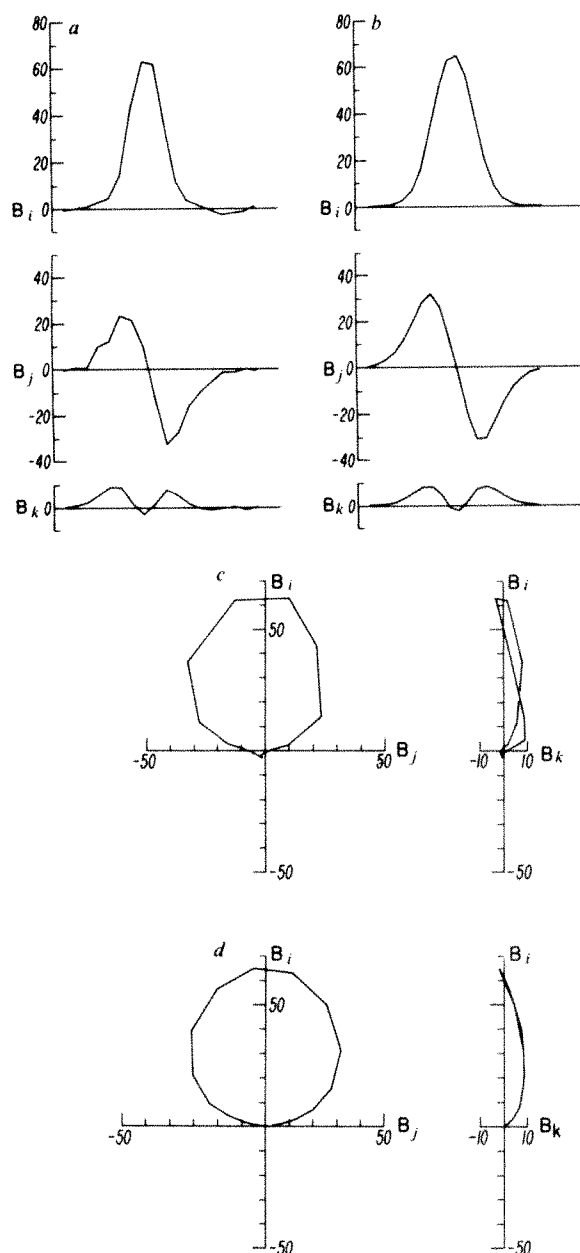
## Observation of magnetic flux ropes in the Venus ionosphere

MAGNETIC field measurements made by the Pioneer Venus Orbiter spacecraft reveal a very low average field strength within the dayside ionosphere of Venus, typically only a few nanoteslas, in contrast to fields of several tens of nanoteslas just outside the ionosphere<sup>1</sup>. Thus, at least in the range of solar zenith angles ( $65-90^\circ$ ) initially probed by the orbiter, the compressed interplanetary magnetic field of the shocked solar wind plasma (the magnetosheath) is effectively shielded from the ionosphere by currents flowing on the ionopause, the boundary between the ionosphere and the magnetosheath. In addition, the magnetic field pressure just outside the ionopause approximately balances the ionospheric thermal plasma pressure<sup>2,3</sup>. However, within this generally low-field region the spacecraft occasionally passes through regions of very large field strength which can sometimes exceed that observed external to the ionosphere. These intense, short-lived enhancements are described here and interpreted to be due to the passage of the spacecraft through 'flux ropes', bundles of twisted magnetic field lines surrounded by ionospheric plasma.



**Fig. 1** Magnetic field enhancements observed within the ionosphere shortly after periapsis (at 1431.56) on orbit 3 of Pioneer Venus. Before and after this interval, the characteristic ionospheric field strength is less than 10 nT, while the peak field just outside the ionopause is  $\sim 55$  nT.





**Fig. 2** *a, b* Comparison of the magnetic structure of one of the enhancements shown in Fig. 1 (*a*), and a model of the enhancement (*b*), the coordinate system is the principal axis system given by minimum variance analysis, and the fields are in nanoTeslas. The model parameters are discussed in the text. 13.5 s of data are shown. Hodograms of the structure (*c*) and the model (*d*). Orbit 3 of Pioneer Venus; 7 December 1978; 1432.37.3–1432.50.8;  $l_A = 3.2$ ;  $l_B = 4.5$ ;  $\rho_{\min} = 2$ .

Figure 1 shows the magnetic field strength observed near periapsis on the third orbit of Pioneer Venus. The spacecraft moves from an altitude of 209 km and a solar zenith angle of  $66.7^\circ$  to an altitude of 332 km and an angle of  $69^\circ$  during this time. Numerous enhancements occur throughout the ionospheric passage, and the field components (not shown) undergo complicated variations within each enhancement. It is unlikely that these variations are purely temporal, as the spacecraft is travelling faster than plausible wave disturbances. For example, with local ionospheric densities  $>10^4 \text{ cm}^{-3}$ , temperatures of roughly 2,000 K (refs 2, 3), and a background field of roughly 2 nT, the Alfvén speed is  $\sim 0.5 \text{ km s}^{-1}$ , while the sound speed is  $\sim 1 \text{ km s}^{-1}$ . The orbital speed of the spacecraft near periapsis is

$\sim 10 \text{ km s}^{-1}$ , so we are probably observing spatial structures. Because the spacecraft grazes these structures at various distances from their centres, the magnitude and duration of the enhancements are variable, as can be seen in Fig. 1. This figure also shows that structures often overlap one another, and for clarity, we have analysed only distinctly separate enhancements.

In an attempt to reduce the field variation to two dimensions, we have performed minimum variance analyses<sup>4</sup> on some of these enhancements. Figure 2*a* shows the field variation, in the principal axis system, of the structure observed between 1432.37.3 and 1432.50.8 UT on orbit 3. The *i*, *j* and *k* components refer to the maximum, intermediate and minimum variance directions respectively. Figure 2*b* shows the results of a minimum variance analysis applied to a passage through a model field describable by the following equations:

$$B_\phi = B(\rho) \times \cos(\alpha(\rho)),$$

$$B_z = B(\rho) \times \sin(\alpha(\rho))$$

where

$$B(\rho) = B_0 \times \exp(-\rho^2/l_B^2)$$

and

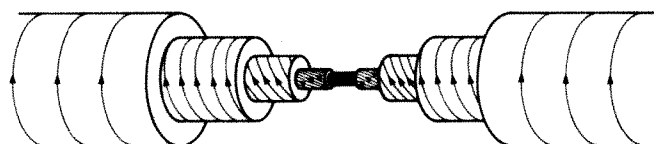
$$\alpha(\rho) = \pi/2 \times (1 - \exp(-\rho^2/l_A^2))$$

These equations define a helical magnetic field, whose magnitude and pitch  $\alpha$ , depend on the radial distance,  $\rho$ , from the axis of the structure.  $B_\phi$  and  $B_z$  are, respectively, the azimuthal and axial components of the field. The scale lengths  $l_A$  and  $l_B$  determine how rapidly the pitch and magnitude vary with distance from the axis. For the case shown in Fig. 2*a, b*  $l_A = 3.2$ ,  $l_B = 4.5$ , and the distance of closest approach to the axis was 2.0. Hodograms of both the observations and the model are shown in Fig. 2*c, d*; the field vector swings through a full  $180^\circ$  in the *i-j* plane, passing through maximum magnitude near  $90^\circ$ . The distinctive variation in  $B_k$  is a result of passing through the helical structure off-axis.

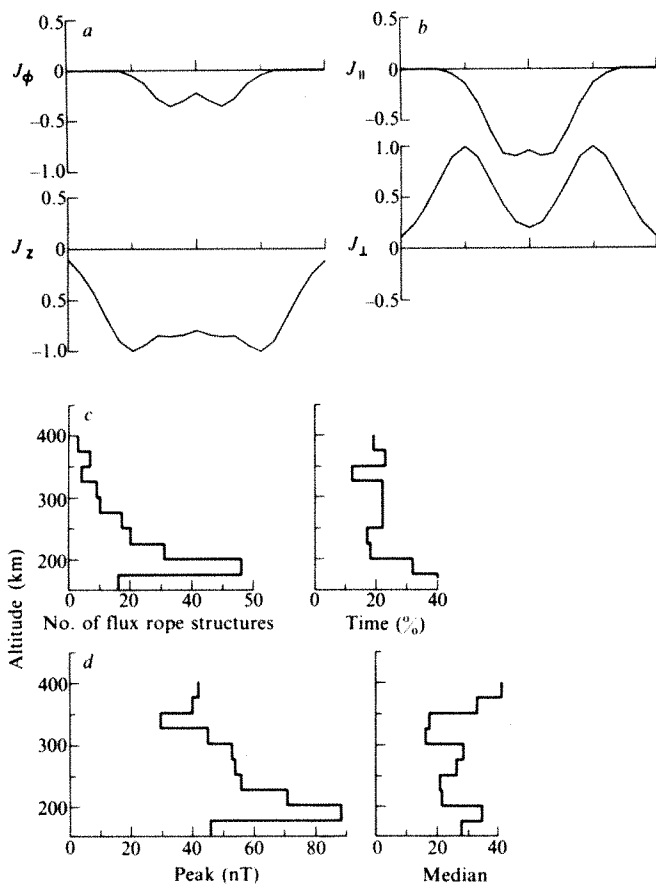
The magnetic configuration suggested by the data and described explicitly by the model is shown in Fig. 3. Because of the helical nature of the magnetic field, the structure resembles a rope of twisted magnetic flux tubes: the magnetic field is axial and at its peak strength in the centre of the structure, becoming weaker and more azimuthal with increasing distance from the axis.

The currents which produce this helical magnetic structure are shown in Fig. 4*a, b*, both in the cylindrical coordinates of the structure and in coordinates parallel and transverse to the magnetic field at each point. Note that throughout the modelled passage the axial current is always dominant.

To determine the source and evolution of these flux ropes, we next examine their amplitude and occurrence frequency with altitude. Figure 4*c, d* shows these quantities combined for orbits 3, 4, 6 and 7. The altitude range is divided into 25-km bins, and the total number of flux rope structures per bin is shown in Fig. 4*c*. Because of its orbit, the spacecraft spends more time in the low-altitude bins than in the high, and this effect can be seen as an increase in total number of structures per bin with decreasing altitude. The altitude interval 150–175 km was sampled only during orbit 7, and does not have an observing time comparable to the higher altitude bins. To correct for the varying observing time with altitude we have determined the ratio of the total time spent within flux ropes to the total time spent within an altitude bin, as shown in Fig. 4*c*. There seems to be a slight increase in



**Fig. 3** Inferred magnetic structure of an ionospheric flux rope. The field is weak and azimuthal in the outer regions, becoming much stronger and more axial near the centre of the structure.



**Fig. 4** *a, b* Current densities (arbitrary units) of the model structure in Fig. 2*a, b*. The cylindrical components (*a*)  $J_\phi$  and  $J_z$  refer to the azimuthal and axial current contributions, while  $J_\parallel$  and  $J_\perp$  (*b*) are the contributions parallel and transverse, respectively, to the local magnetic field vector. The abscissa is distance along the spacecraft trajectory which in this case does not pass through the centre of the rope. Distributions of occurrence frequency (*c*) and amplitude (*d*) with altitude.

occurrence rate at lower altitudes. Additional data, especially when Pioneer Venus returns to the dayside ionosphere, will be needed to confirm this tendency.

If the magnetic field in these structures is in pressure balance with the surrounding ionospheric plasma, one would expect their characteristic field strength to increase with decreasing altitude, as the plasma thermal pressure increases. Figure 4*d* shows the peak flux rope amplitude observed within each altitude bin, and indeed, the amplitude seems to increase with decreasing altitude. The 150–175 km bin is the most notable exception to the trend, perhaps because this lowest altitude interval was sampled only on orbit 7. The right portion of Fig. 4*d* shows the median of the distribution of flux rope amplitudes within each bin. The distribution suggests that, at altitudes below 350 km, amplitudes increase overall with decreasing height, but results above 325 km have less statistical accuracy because of the small number of samples in those intervals.

Although the results shown are only based on four orbits of data they, nevertheless, carry implications for the source and evolution of flux ropes. First, the suggestion that these structures are more numerous at lower altitudes may be due to either a 'piling up' effect or to a source at low altitudes. The former might occur if magnetosheath magnetic flux tubes were pulled into the ionosphere by solar wind convection, such as has been proposed for the terrestrial magnetopause<sup>5</sup>. These structures would slow down and pile up in the denser, slower moving lower ionosphere. If, however, these structures result from a source in the

lower ionosphere, they might percolate upwards due to buoyancy; indeed, the enhanced magnetic field pressure within these structures implies the reduction of the plasma thermal pressure there, and if this shows up as a reduction in ion/electron number density, flux ropes must be buoyant with respect to the surrounding plasma. A similar process is believed to occur in the terrestrial equatorial ionosphere<sup>6</sup>. The helical nature of flux ropes could develop from velocity shear in the ionosphere, due perhaps to a viscous interaction with the magnetosheath at the ionopause. Given the observed ionospheric temperatures and densities, and flux rope scale diameters between 10 and 100 km, the magnetic diffusion time of such structures would be many hours. Thus flux ropes are likely to persist long enough to be transported, not only from the ionopause to the lowest ionospheric levels (or vice versa), but from the subsolar region to the terminator as well.

Whatever their source, these ionospheric flux ropes may play an important part in the solar wind–ionosphere interaction, and ionospheric plasma transport and heating. Analysis of the early Pioneer Venus data is continuing to define better the nature of this unexpected phenomenon.

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## Polar heating and the shape of Venus

ON Venus, as on the other terrestrial planets, temperature variations are expected between equator and poles and between day and night. Several attempts have been made to measure these deviations from circular symmetry in the radio emission from Venus using interferometric techniques, for example, Berge and Greisen<sup>1</sup> at a wavelength of 3.1 cm and Sinclair *et al.*<sup>2–4</sup> at 11 cm. The observed variations are found to be very small. Berge and Greisen<sup>1</sup> report a slight increase in the brightness temperature towards the poles, but the effect was less than one standard deviation of the measurements. A larger increase in brightness temperature towards the poles was seen by Sinclair *et al.*<sup>4</sup>. We report here a similar polar brightening at a wavelength of 6 cm which persuades us that the effect is real. This brightening is a surface or near surface phenomenon as at 6 cm about half the flux is from the surface and half from the lower atmosphere.

Our observations were made with a two-element interferometer at Owens Valley Radio Observatory between 6 May and 13 May 1977, near inferior conjunction with Venus. Each element of the interferometer is a steerable, parabolic, 27-m diameter antenna with a linearly polarised feed that can be rotated by remote control. The polarisation of the E-vector accepted from Venus was either perpendicular or parallel to the baseline projected on the planet and was alternated between successive records. The observational records were 5 min long with calibration records every 45–60 min. Small-diameter extragalactic radio sources with accurately known positions were used to calibrate the fringe amplitude and phase.

An east–west (E–W) baseline of 396 m and a north–south (N–S) baseline of 456 m were selected to observe near the first

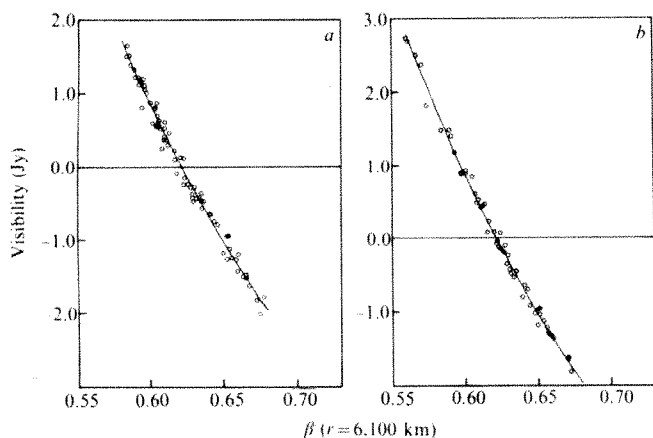
null of the planet's 6-cm visibility function, which is the Fourier transform of the spatial brightness temperature distribution. These baselines were nearly parallel to N-S and E-W directions on Venus, as well as on the Earth. The resulting visibility functions are most conveniently expressed as a function of  $\beta$ , the instantaneous projected baseline length in wavelength times the angular radius of the planet based on a physical radius of 6,100 km. The value of  $\beta$  at the first null of the visibility function,  $\beta_0$ , varies inversely with the width of the brightness-temperature distribution along the baseline. Thus, by comparing the E-W and N-S values of  $\beta_0$ , the directions in which the planet is wider and/or hotter towards the limbs can be determined. We cannot uniquely deconvolve variations in the planet's radius and temperature variations. Our observations were all near the first null and determine  $\beta_0$  quite accurately.

An interferometer measures both the amplitude of the visibility function and the phase. Theoretically, phase data can be used to detect asymmetries about a line through the centre of the disk perpendicular to the baseline. However, our phase data from the N-S baselines were affected by antenna pointing errors caused by strong winds, and we could not determine the amount of asymmetry between the north and south poles on Venus. To a close approximation, such pointing errors do not affect the measurements of  $\beta_0$ . Sinclair *et al.*<sup>4</sup> report that the pole-to-pole asymmetry is <3 K at a wavelength of 11 cm. Consequently, we have assumed that equator-pole variation is symmetric, that is, that the brightening occurs equally at both poles. Observations on our E-W baseline were not affected by pointing errors as the winds at this time were low.

Figure 1 shows our measurements with polarisation perpendicular to the baseline for the E-W and N-S baselines. To find the best fit value for  $\beta_0$ , we used least squares to fit the quadratic formula,  $B(\beta - \beta_0) + C(\beta - \beta_0)^2$ , to the data for a series of values of  $\beta_0$ . Because a quadratic is a good approximation of the true visibility function over only a limited range of  $\beta$ , we consider only data points with  $0.56 < \beta < 0.68$  for the perpendicular polarisation. We determine the value of  $\beta_0$  that minimises the sum of the squares of residuals:

$$S = \sum_i (f_i - [B(\beta_i - \beta_0) + C(\beta_i - \beta_0)^2])^2$$

where  $f_i$  = data point at  $\beta_i$  and  $B$  and  $C$  are the best fitting coefficients for each value of  $\beta_0$ . The best fitting values for  $\beta_0$  are  $0.6207 \pm 0.0005$  for N-S<sub>⊥</sub> and  $0.6214 \pm 0.0004$  for E-W<sub>⊥</sub>, where the errors are standard deviations determined from the



**Fig. 1** Visibility data for *a* N-S and *b* E-W baselines with polarisation perpendicular to the baseline. *a*,  $\beta_0 = 0.6207 \pm 0.0005$ ; *b*,  $\beta_0 = 0.6214 \pm 0.0004$ . The visibility is plotted plus or minus according to the phase. The curves shown and the values given for  $\beta_0$  were determined by a least-squares fit of a quadratic as described in the text. The radius used in calculating  $\beta$  was 6,100 km.

data. The difference in nulls  $\Delta\beta_0$ , is  $0.0007 \pm 0.0006$ , where the error again is the standard deviation.

Our measurements with polarisation parallel to the baseline are shown in Fig. 2. The best fit values over the range  $0.55 < \beta < 0.67$  for  $\beta_0$  are  $0.6075 \pm 0.0005$  for N-S<sub>||</sub> and  $0.6082 \pm 0.0004$  for E-W<sub>||</sub>. The parallel nulls are at smaller values of  $\beta_0$  than the perpendicular nulls because of a maximum in the brightness in this polarisation at the Brewster angle. The difference in nulls for the parallel polarisation is also  $0.0007 \pm 0.0006$ .

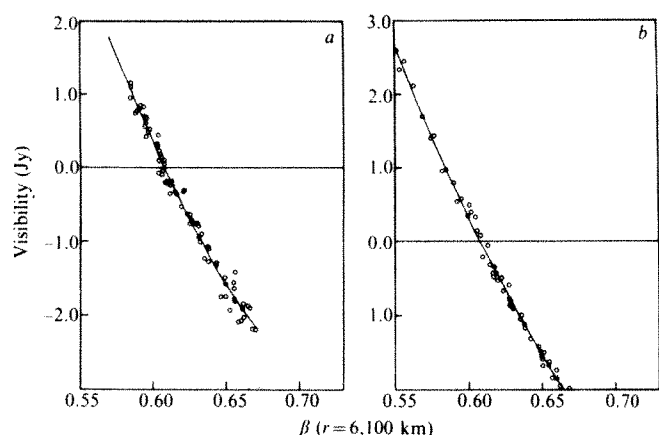
As the measurements in the two polarisations are statistically independent, we may combine our results to get  $\Delta\beta_0 = 0.0007 \pm 0.0004$ . Note that in both polarisations the N-S  $\beta_0$  is less than the E-W  $\beta_0$ . This difference implies that the planet is either larger or brighter at the limb along the N-S direction than along the E-W direction. This result is surprising. If we assume that the  $\Delta\beta_0$  is produced by a variation in radius of a uniformly bright planet, the planet's figure must be  $7 \text{ km} \pm 4 \text{ km}$  prolate. If the planet's gravitational equipotentials are prolate to this extent as well, the resulting  $J_2$  is about  $-10^{-3}$ , but  $J_2$  for Venus has been estimated to be  $-2 \pm 5 \times 10^{-6}$  by Esposito (personal communication) and  $4.0 \pm 1.5 \times 10^{-6}$  by Akim *et al.*<sup>5</sup>

It is difficult to compare our results with the polar heating reported by Sinclair *et al.*<sup>4</sup>, because they do not report a model-independent measure of the effect such as the prolateness of an equivalent, uniformly bright elliptical disk. Sinclair *et al.* altered their standard circularly symmetric Venus model to include a latitudinal surface temperature variation of the form  $\Delta T \sin^2 \varphi$  where  $\varphi$  is the latitude. The form  $\sin^2 \varphi$  was chosen as it is the first even term of a harmonic expansion. The resulting surface temperature at each latitude was adiabatically extrapolated upwards through the atmosphere. Visibility functions computed from this model were compared with their measured visibilities and  $\Delta T = 16 \pm 2.5 \text{ K}$  was estimated. Apparently, the quoted error does not include any model uncertainties. A similar procedure with our data would yield a  $\Delta T$  of about 5 K. The magnitude of the effect may be variable.

There are several viable phenomena that would cause the poles of Venus to be hot or to appear hot at radio wavelengths, although the thermal time constant in the lower venusian atmosphere is very long and, on theoretical grounds, one expects nearly constant temperatures on any equipotential surface. The absence of appreciable E-W asymmetries in the radio brightness across the terminator<sup>4</sup> strongly supports this idea. However, the global atmospheric circulation may cause relative heating towards the poles. There may be strong downwelling causing warming associated with the circumpolar vortex<sup>6</sup>. IR measurements on the Pioneer Venus Spaceprobe<sup>7</sup> detected a brightening of  $\sim 10 \text{ K}$  over the north pole, down to an altitude level of about 70 km. These measurements are only sensitive to the atmospheric temperatures above the clouds. This region contributes nearly zero flux at radio wavelengths. It is unlikely that these elevated temperatures above 70 km are related to the brightening at the surface. A second possible cause of polar heating is a slight topographical flattening relative to the gravitational equipotentials possibly due to a more rapid spin state in the past or to the dynamic alignment of the planet's rotation axis and its axis of greatest moment of inertia. The latter case would apparently also require a more rapid rotation in the past. It is also possible that topography generated by geological processes could coincidentally cause this effect. Two phenomena that would increase the radio brightness towards the poles without increasing the physical temperature are a systematic decrease in radio opacity caused by a thinning of the clouds towards the poles or a systematic increase in surface emissivity away from the equator due to latitudinal differentiation of the surface geology.

The possibility of a greater topographic than gravitational oblateness is explored below. Atmospheric circulation will be considered elsewhere. It is not unreasonable to expect Venus's figure to have a small excess topographic oblateness. The Earth's figure has an excess oblateness of about 0.5 km, primarily because a majority of the continents are located nearer





**Fig. 2** Visibility data for *a* N-S and *b* E-W baselines with polarisation parallel to the baseline. *a*,  $\beta_0 = 0.6075 \pm 0.0005$ ; *b*,  $0.6082 \pm 0.0004$ . Otherwise as Fig. 1.

the Equator than the poles<sup>8</sup>. The Moon and Mars also have excess oblateness of about the same magnitude<sup>9,10</sup>.

Model calculations were carried out to determine how large a bulge is required to explain our observations. In the model, the figure of the planet is assumed to be an oblate spheroid. In general, the gravitational equipotentials are oblate also due to the oblate figure and rotation. However, if the planet is not in hydrostatic equilibrium, the topographical oblateness will exceed the gravitational oblateness unless the density distribution is prolate. For simplicity in calculations, the gravitational equipotentials in the model are assumed to be spheres. The atmosphere is well mixed so the surfaces of constant temperature and pressure are also spherical. The poles are lower in the atmosphere than the equator and are, therefore, warmer. An atmospheric model and a computational method similar to that of Muhleman *et al.*<sup>11</sup> are used to calculate brightness temperatures which are integrated numerically over the planet's disk to find the model visibility function for an E-W and a N-S baseline. In calculating brightness temperatures, the effects of absorption, emission and refraction in the atmosphere are considered along with the effects of reflection and emission by a dielectric surface. We find that a topographical oblateness of  $0.6 \text{ km} \pm 0.4 \text{ km}$  will yield a  $\Delta\beta_0$  that agrees with our observations. We believe that this is a good approximation of the amount by which the topographical oblateness must exceed the gravitational oblateness. This amount of oblateness together with the  $7.8^\circ \text{ km}^{-1}$  lapse rate leads to the poles being  $5^\circ \pm 3^\circ$  warmer than the equator.

To determine whether the excess topographical oblateness could be due to a fossil rotational bulge, we use the model of Melosh<sup>12</sup> for a despun planet. The structure of the planet in this model is an elastic shell over a fluid interior. We assumed an initial rotation period of 1 day and a density contrast between the interior and the elastic shell of 1.5–2.0. We find that when the excess topographical oblateness is  $0.6 \pm 0.4 \text{ km}$ , the gravitational field generated has a  $J_2$  an order of magnitude above the observational limit. These results indicate that the presence of a fossil rotational bulge on Venus large enough to explain our observations is unlikely. However, the polar heating could still be due to regional isostatically compensated changes in the topography which would have less impact on  $J_2$ .

In conclusion, our observations are consistent with previous work that has shown that the disk of Venus is nearly circularly symmetric at radio wavelengths. However, a small amount of polar brightening is present. We cannot yet determine the cause of this phenomenon although we have argued against the possibility of a fossil rotational bulge large enough to explain the result. As the poles of Venus have other interesting aspects involving atmospheric temperatures and circulation, they are an area worthy of future investigation.

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## X-ray, optical and radio observations of A1710–34

SAS 3 ROTATING modulation collimator (RMC) measurements are reported here which yield a new, more precise, position for the Ariel 5 X-ray source A1710–34 (ref. 1). We also report optical observations of stars in the X-ray error box and discuss evidence obtained with the Parkes 64 m radio telescope for 2-cm radio emission from the vicinity of the X-ray source.

Measurements were made of A1710–34 with the 2.3' and 4.5' FWHM RMC detectors on the SAS 3 X-ray observatory<sup>2,3</sup>. These detectors are sensitive in the range 2–11 keV. Two independent sets of position measurements were made between 1600 UT 16 July and 1200 UT 22 July 1978. Details of the observations and the most probable position for the source (which we designate 2S1711–339) are given in Table 1. This position is 2.2 arc min from the most probable position determined using the Ariel 5 satellite and is therefore just inside the Ariel 5 error box. The 90% error circle of the new position has a radius of 40 arc s. A finding chart from the SRC Schmidt Survey J-plate is presented in Fig. 1.

The error circle for this observation of 2S1711–339 includes seven stars of the European Southern Observatory (ESO) quick blue survey plate with magnitude,  $m_{\text{pg}} \leq 20$ . The brightest star (number 1, Fig. 1) has a magnitude,  $m_{\text{pg}} \sim 15$ . The Anglo-Australian Telescope and the RGO spectrograph (140 Å mm<sup>-1</sup> dispersion) were used to obtain a spectrum (3,200–7,000 Å) of this star. The star is of spectral type G0 and has no perceived emission lines. We therefore have no spectroscopic evidence to suggest that it is the optical counterpart to the X-ray source.

The SRC-J survey plate shows that the X-ray error circle lies in the direction of a dust lane. Absorption is particularly strong in the eastern half of the error circle. If the X-ray low energy spectrum is heavily cut off, the optical candidate is more likely to be faint and to the east behind the dust lane. Low-energy X-ray observations would be useful to clarify this particular point. No particularly blue or red stars or variables could be identified by comparing ESO (blue) and SRC (yellow) survey plates.

**Table 1** RMC observations of A1710-34 (2S1711-339)

Date (1978)	Exposure	Collimator	RA (1950)	Dec (1950)	Intensity $\mu\text{Jy}$ (2-11 keV)
0700 UT 20 July	49,500 s	2.3' FWHM	257.7579	-33.9897	$13.2 \pm 2.2$ (6 $\sigma$ )
1200 UT 22 July			17 h 11 min 1.9 s	-33°59'23"	
0700 UT 20 July	49,500 s	4.5' FWHM	257.7500	-33.9978	$14.6 \pm 2.3$ (7 $\sigma$ )
1200 UT 22 July			17 h 11 min 0.0 s	-33°59'52"	
1600 UT 16 July	74,000 s	2.3' FWHM	257.7583	-33.9908	$16.6 \pm 2.6$ (7 $\sigma$ )
0500 UT 20 July			17 h 11 min 2.0 s	-33°59'27"	
1600 UT 16 July	74,000 s	4.5' FWHM	257.7554	-33.9908	$15.3 \pm 2.2$ (7 $\sigma$ )
0500 UT 20 July			17 h 11 min 1.3 s	-33°59'27"	
Average			257.7554	-33.9922	$14.9 \pm 1.2$
			17 h 11 min 1.3 s	-33°59'32"	

**Table 2** Summary of X-ray, radio and optical observations of A1710-34

Waveband	Position (1950.0)		Error circle radius (90% confidence)	Intensity
	RA	Dec		
X-ray (2-11 keV)	17 h 11 min 1.3 s	-33°59'32"	40 arc s	$14.9 \pm 1.2 \mu\text{Jy}$
Radio (2 cm)	17 10 52	-34 00 36	2.2 arc min	$23 \pm 5 \text{ mJy (max)}$
Optical (star 1)	17 11 1.32	-33 59 12.8	2 arc s	$m_{\text{pg}} \sim 15$

The region of the sky in the vicinity of A1710-34 (2S1711-339) has been searched for 2-cm radio emission using the Parkes 64m radio telescope with a full width to half-power beam of 2.2 arc min. Dual-beam switching between two identical beams separated by 6.5 arc min was used. The measurements were made in June 1977, August 1977 and April 1978 during a comprehensive search<sup>4</sup> for new radio counterparts of X-ray sources using Ariel 5, SAS 3 RMC and HEAO 1 positions. The new X-ray position was not known at the time of these observations.

No significant signal was detected from the vicinity of A1710-34 (2S1711-339) in June 1977 or April 1978. Two-sigma upper limits were 66 and 12 mJy respectively. A flux density of  $23 \pm 5 \text{ mJy}$  was detected in August 1977. As the

source was too weak for a reliable radio position determination, the beam was pointed at the Ariel 5 RMC source position. The sensitivity to a point source at the centre of the SAS 3 X-ray error circle would be  $\sim 13\%$  of the on-axis response.

Radio counterparts of galactic stellar X-ray sources tend to be very variable. Thus, for example, the radio emission from Sco X-1 varies in an irregular manner with a typical time scale of hours. The range of variability is about two orders of magnitude<sup>5</sup>. GX9+1 and GX17+2 exhibit short term variability of the Sco X-1 type<sup>6</sup>. Circ X-1 is also variable on a time scale of hours<sup>7</sup>.

We have applied the  $\chi^2$  test to the hypothesis that the radio signal seen in August 1977 could have occurred by chance from a source of constant flux density equal to the weighted mean of the three observations. This gives  $\chi^2 = 7.7$  for 2 d.f. The probability of obtaining a value of  $\chi^2 \geq 7.7$  is about 2%. It therefore seems reasonable to assume that A1710-34 (2S1711-339) is a variable radio emitter with an intensity which rises above the threshold of the Parkes radio telescope at 2 cm only during periods of major flaring.

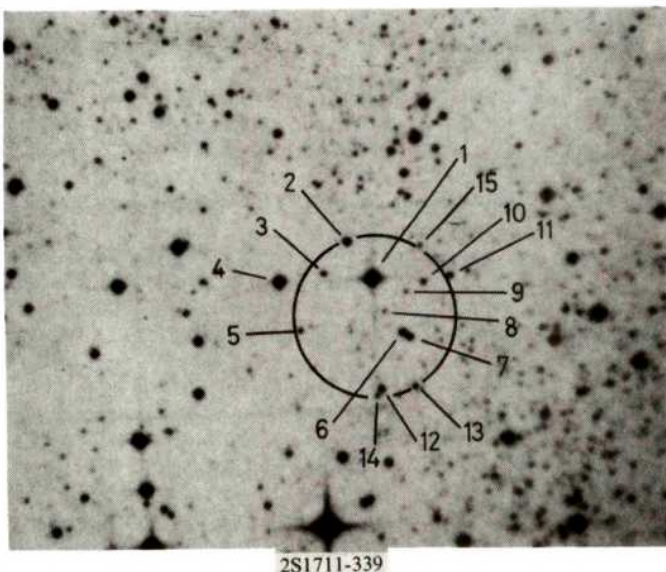
We conclude that there is probably a variable 2-cm radio source in the vicinity of A1710-34 (2S1711-339) but that further observations are required to determine the position accurately. In Table 2 we summarise the observations made in the three different wavelength bands.

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**Fig. 1** Finding chart for the SAS 3 RMC position for 2S1711-339, taken from the SRC Schmidt Survey J-plate. The scale is given by the diameter of the error circle which is 80 arc in diameter. North-east is in the upper left-hand corner.

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## On the detection of jovian companions to white dwarfs

BRACEWELL<sup>1</sup> has recently proposed detection of planetary companions to nearby stars by means of a spinning IR interferometer. In particular, he notes that at far IR wavelengths, the emissivity ratio of the planet to the star increases by five orders of magnitude over equivalent values in the visible. I point out here that more than another three orders of magnitude in sensitivity is gained by choosing white dwarfs as the observational objects, which may permit detection of jovian or black dwarf companions by direct photometry from a space telescope with photon-limited, far IR detectors.

Compared with the Sun, a typical DA white dwarf has a surface area only  $1\text{--}2 \times 10^{-4}$  as great, while its photospheric temperature is approximately double. Thus, at wavelengths in the Rayleigh–Jeans regime, an observer would detect 2,500–5,000 times fewer photons from a white dwarf than from a solar-type star at the same distance. The key assumptions to this proposal then are that planets of comparable or greater than jovian mass will (1) survive the parent star's red giant phase, and (2) presently contain internal energy sources similar to that of Jupiter. I discuss each of these assumptions in turn.

The most serious threat to a planet's survival is engulfment by the red giant photosphere. Viscous drag on the planet's orbital motion would then lead to a spiralling into the stellar core. The maximum luminosity<sup>2</sup> of a red giant with a  $1.4 M_{\odot}$  carbon-oxygen core (that is the Chandrasekhar limit) is  $\sim 5.2 \times 10^4 L_{\odot}$ . If the red giant's effective temperature is  $\geq 2,500$  K, all planets with orbital semi-major axes  $> 5.7$  AU should remain outside the photosphere. For a  $1.0 M_{\odot}$  core, the equivalent value is 4.2 AU. Runaway atmospheric evaporation might also cause planetary destruction. However, Jupiter's atmosphere must be heated to temperatures far above 3,000 K ( $GM_p/kR_p \sim 180,000$  K) before significant quantities of atomic hydrogen will escape. Therefore, as long as a jovian planet remains outside the red giant photosphere, it will probably survive and perhaps even grow slightly from stellar wind accretion.

Both Jupiter and Saturn have internal energy sources. For Jupiter, this source provides more than half its observed thermal emission. Though gravitational contraction may be partially responsible, most astronomers favour an explanation relying on the radiation of primordial heat of formation. Graboske *et al.*<sup>3</sup> have calculated the initial contraction and subsequent evolution of a  $0.0095 M_{\odot}$  protoplanet and found that after a relatively short time ( $O(10^6 \text{ yr})$ ) spent along the Hayashi track, a rapid transition to the cooling curve of a very cold degenerate dwarf occurs. In this latter regime,  $\log L/L_{\odot}$  scales as  $-1.3 \log t$ . As the average age of a white dwarf (including its progenitor's lifetime) is somewhat greater than half that of the Galaxy, the present

luminosity of a hypothetical jovian companion should remain at approximately half its value at  $t = 4.6 \times 10^9 \text{ yr}$ , the age of our Solar System. Thus, one expects relatively strong internal emission to be a general property of all planets of jovian mass or greater.

The three or more orders of magnitude gained in relative sensitivity by the choice of white dwarfs rather than solar-type stars suggest that planetary companions might be detected as an 'IR excess' if advanced detectors become available. Adopting sample parameters of  $\lambda = 40 \mu\text{m}$ ,  $\Delta\lambda = 10 \mu\text{m}$ ,  $R_{\text{WD}} = 0.013 R_{\odot}$ ,  $R_p = 0.1 R_{\odot}$ ,  $T_{\text{WD}} = 10^4 \text{ K}$ ,  $T_p = 120 \text{ K}$ , and a distance of 10 pc, a collecting area of  $10^4 \text{ cm}^2$  would receive  $\sim 180$  photons  $\text{s}^{-1}$  from the white dwarf and  $\sim 20$  photons  $\text{s}^{-1}$  from the planet. A hypothetical photon-limited detector above the Earth's atmosphere could reveal the excess planetary signal with a signal-to-noise ratio  $> 10$  in less than a minute. Confusion with the relic circumstellar dust shell of the red giant is unlikely because it should have been quickly swept away by the ram pressure associated with the white dwarf's motion through the interstellar medium. Zodiacal dust, however, may lead to serious background noise limitations<sup>1</sup> and increase detection times for a diffraction limited telescope by as much as a factor of 1,000 at low ecliptic latitudes.

One might also detect or set upper limits on the number of substellar, degenerate 'black dwarfs'<sup>4</sup> in the mass range  $10^{-3}$  to  $\sim 0.06 M_{\odot}$  associated with white dwarfs. Such objects may provide an important contribution to the mass density, both locally and in clusters of galaxies<sup>5,6</sup>. Their IR luminosity should scale at least linearly with mass. The advantage of a search in the immediate vicinity of a white dwarf is simply that one has a quite definite, highly localised position on the sky to measure. For a background-limited detection system, this allows one to maximise the signal-to-noise ratio until either the diffraction limit or the effective 'seeing' limit is reached.

There are over a dozen single white dwarfs known within 10 pc of the Sun<sup>7</sup>. A far IR survey of these stars from a space-borne telescope might quickly reveal whether solar system formation was a common event. A positive answer would give great impetus towards the construction of a more elaborate planet-detector, such as that proposed by Bracewell.

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## Do comets provide material for the anomalous component of the cosmic rays?

MATTER from comets is proposed here to form a significant part of the source material for the 'anomalous component' of the cosmic rays. As a result it is concluded that certain molecular ions, such as  $\text{CO}^+$ , should be present at energies around 10 MeV per nucleon.

The spectra of the nucleonic component of cosmic radiation in the energy band 1–60 MeV per nucleon have produced a number of surprises. The most dramatic of these concerned the fluxes of oxygen and carbon. Jokipii<sup>1</sup> gave minimum flux values in the region 5 MeV per nucleon for hydrogen and helium which essentially divided the solar particles on the low energy side from the true cosmic radiation at the higher energies. The



Chicago group reported in 1973<sup>2</sup> that the shape of the helium spectrum had changed in that the minimum reported earlier had now been filled in, whereas the hydrogen spectrum was essentially unchanged. Hovestadt *et al.*<sup>3</sup> also detected in 1973 an anomalous flux of oxygen centred around 5 MeV per nucleon; as there was no corresponding flux of carbon the C/O ratio had changed by a considerable factor. Later experiments confirmed these measurements and showed the oxygen flux to be 20 times the value of the carbon flux at this peak. This is in complete contrast to the situation below 1 MeV per nucleon or above 60 MeV per nucleon where the C/O ratio remained close to unity. The later measurements also showed a substantial enhancement of nitrogen, and indicated a likely enhancement of neon. The total flux of the heavier elements is  $\sim 1.0 \text{ m}^{-2} \text{ sr}^{-1} \text{ s}^{-1}$ , in the band  $3 < E < 60 \text{ MeV}$  per nucleon and that of helium is a few times larger.

The discovery of the anomalous component was very surprising and demanded an explanation. The generally accepted explanation is due to Fisk, Kozlovsky and Ramaty<sup>4</sup> who postulate that these particles carry only a single charge in contrast to both the solar corpuscular radiation at  $\sim 1 \text{ MeV}$  per nucleon and the cosmic rays at higher energies which are fully stripped. The consequently high magnetic rigidity ( $\sim 2 \text{ GV}$ ) is an essential feature of their explanation. They envisage the following stages: (1) The passage of the Solar System through the local interstellar gas sweeps aside charged ions, while the neutral atoms are able to penetrate the magnetic cavity of the Sun. A fraction of these atoms is ionised well within the solar cavity. Species likely to be uncharged in interstellar space include He, N, O, Ne since their first ionisation potential is higher than that of the interstellar hydrogen which shields them. (2) After ionisation the singly charged species are carried by magnetic disturbances out to  $\sim 50 \text{ AU}$  where magnetic inhomogeneities accelerate them preferentially, owing to their high rigidity. They retain their unit charge; the mean lifetime for further ionisation will be in the range  $10^6$ – $10^8 \text{ s}$ , depending principally on their distance from the Sun. (3) After acceleration the ions, still presumably singly charged, diffuse through the Solar System, losing little energy from adiabatic deceleration. Again this is due to their rigidity being so much higher than cosmic ray and solar species of comparable energy that are fully stripped.

This model accounts for the particular enhancement of He, N, O and Ne atoms in terms of the magnitude of their first ionisation potential. The ions are introduced to the acceleration mechanism by being ionised well within the solar cavity. The onset of the anomalous component in 1973 and its continuation to the present is presumed to be concerned with the solar cycle and its effect on the acceleration mechanism.

If stages (2) and (3) operate on singly charged ions, clearly any other source of such ions might well contribute to the anomalous component. Comets seem to be such a source. The coma consists of neutral atoms and molecules which are the sublimation products from the cometary nucleus. The most abundant species in the coma seem to be degradation products of  $\text{H}_2\text{O}$ . In addition, in most comets, ionised atoms and molecules are swept by the solar wind into the familiar cometary tail. In this ion tail the spectrum from  $\text{CO}^+$  is usually the most prominent component. Ions liberated by comets could be a direct source of material for stages (2) and (3) above. Further, atoms and molecules from the coma might be an alternative source of neutral particles for stages (1) to (3) above. The presence of molecules in this cometary debris leads us to an interesting possibility. These molecules might also be present in the anomalous component, as in many cases their lifetimes for further ionisation or dissociation have values comparable to those of atomic ions. A cometary source of material would have the following features.

First, the total amount of  $\text{H}_2\text{O}$  shed by short period comets is estimated by Delsemme<sup>5</sup> to be  $\sim 5 \times 10^7 \text{ g s}^{-1}$ . In addition the amount of more refractory material released by comets to form meteoroids that produce the zodiacal light and the Gegenschein is estimated by Whipple and Huebner<sup>6</sup> as  $1$ – $3 \times 10^7 \text{ g s}^{-1}$ . These values are comparable with that coming in from the interstellar

**Table 1** Binding energies of some abundant molecules and molecular ions

	Dissociation energy (eV)	Ionisation energy (eV)		Dissociation energy (eV)	Ionisation energy (eV)
$\text{H}_2$	4.54	15.43	$\text{H}_2^+$	2.71	16.3
CH	3.52	11.13	$\text{CH}^+$	3.65	17.2
$\text{C}_2$	6.27	12.0	$\text{C}_2^+$	5.53	16.8
CN	8.01	14.3	$\text{CN}^+$	4.96	19.5
CO	11.20	14.01	$\text{CO}^+$	8.45	22.1
NH	3.27	13.10	$\text{NH}^+$	3.77	18.3
$\text{N}_2$	9.88	15.58	$\text{N}_2^+$	8.83	23.4
NO	6.57	9.25	$\text{NO}^+$	10.93	25.6
OH	4.45	13.17	$\text{OH}^+$	4.88	18.5
$\text{H}_2\text{O}$	5.17	12.6	$\text{H}_2\text{O}^+$	5.75	19.3
$\text{CO}_2$	5.51	13.77	$\text{CO}_2^+$	5.35	19.0

medium as neutral atoms, which for oxygen is estimated as  $\sim 5 \times 10^7 \text{ g s}^{-1}$ , by Fisk *et al.*<sup>4</sup>

Second, when first ionised the atom or molecule has a velocity and a component of momentum transverse to the magnetic field almost identical with the values possessed by the interstellar atoms.

Third, the position of the maximum release of cometary material is in the region of 1 AU, bracketed by the mean distances reached by neutral He, N and O before ionisation ( $0.5$ – $4 \text{ AU}$ )<sup>7</sup>.

Finally, the chemical composition of comets has several features in common with that of the anomalous particles. The detected spectra suggest that the principal components are degradation products of  $\text{H}_2\text{O}$ . However, contributions from the refractory material could include a greater variety of elements, for example, Mg and Si.

These points indicate that cometary material is apparently a good candidate for the source of at least some part of the anomalous component. The O/C ratio may place a constraint on the proportion of the anomalous component from comets, but cometary abundance values could well encompass the 20:1 ratio that one has from experiments on the anomalous component. One would not, however, expect a significant helium or neon contribution from a cometary source. The helium flux is well established, and has an abundance a few times that of oxygen, but the data for neon are dubious and are based on a very small number of detected particles, and could have been produced by incident molecular ions, such as CO, rather than neon. The presence therefore of inert gases in significant amounts implies that cometary material cannot be the only source of the anomalous component.

Finally, we would like to consider in further detail the possibility of molecular ions being present in the anomalous component. Table 1 lists some properties of appropriate molecules and molecular ions. Note that the stability of molecular ions against dissociation is comparable with their parent molecules, and their ionisation energy averages 1.5 times that of their parents. In practice the longevity of a molecular species in space is determined principally by whether any copiously produced excited states have a reasonably high dissociation probability. In  $\text{O}_2$ , for example, UV absorption in the Schumann–Runge bands produces dissociation, which is a mechanism of considerable consequence, being the first stage of ozone production in the atmosphere. In contrast there are no such  $\text{N}_2$  states abundantly produced and the effective threshold for  $\text{N}_2$  is ionisation. The longevity of certain molecules, when essentially at rest at about 1 AU, has been estimated by Siscoe and Mukherjee<sup>8</sup> and the figures computed are in the range  $10^6 \text{ s}$ .

As any molecular ion has one fewer weakly bound electron than its parent, and since its ionisation energy is  $\sim 1.5$  times higher, its ionisation cross-section will be significantly smaller than that of its neutral parent. This in turn is smaller than the sum of the cross-sections of its atomic components. The cross-sections of atomic C, N, O, F and Ne are  $\sim 10^{-17} \text{ cm}^2$ , and that of

H is  $\sim 3 \times 10^{-18} \text{ cm}^2$ , for ionisation by protons and electrons having  $\beta \sim 0.14$ . Thus all the molecular ion species listed in Table 1 will have ionisation cross-sections close to  $10^{-17} \text{ cm}^2$  when moving with  $\beta \sim 0.14$ . This leads to a partial ionisation rate at 1 AU of  $\sim 4 \times 10^{-7} \text{ s}^{-1}$  (taking the density of solar wind as  $10 \text{ cm}^{-3}$ ). At  $\beta \sim 0.14$  the charge exchange cross-section with  $\text{H}^+$  from the solar wind is very small, while the photoionisation cross-section for molecular ions will be smaller (because of the higher ionisation energies) than that of the parent molecules with  $\beta \sim 0$  that have been considered by Siscoe and Mukherjee<sup>8</sup>. Thus the lifetime of the fast moving molecular ions against break-up is hardly dependent on its velocity and remains at  $\sim 10^6 \text{ s}$  at 1 AU, increasing as the square of the distance from the Sun. We therefore conclude that the likely lifetime of suitable molecular ions, such as  $\text{CO}^+$ , is adequate for a reasonable survival probability through the whole acceleration and diffusion stages proposed by Fisk *et al.*<sup>4</sup>. There is thus a reasonable chance of detectable fluxes of certain molecular ions with energies in the region of 10 MeV per nucleon.

From these considerations we feel that cometary material may have an important role as part of the source material for the anomalous component and that molecular ions from such a cometary source may be present in the anomalous component. The method of particle identification used in instruments to date means that this last point could not be confirmed. The method uses two measurements, the rate of energy loss  $dE/dx$  and the total energy,  $E$ . For a molecule these are proportional to  $\sum Z_i^2$  and  $\sum A_i$  respectively since each component atom has the same incident velocity ( $Z_i$  and  $A_i$  are the atomic number and mass of the  $i$ th component). Thus  $\text{OH}^+$  closely resembles  $\text{O}^+$ , and  $\text{CO}^+$  will lie between  $\text{Ne}^+$  and  $\text{Na}^+$  in the data from these detectors. The most clearcut way of establishing that an individual event is a molecular ion involves the determination of  $dE/dx$  at several different energies covering the whole range of a stopping particle. For molecules consisting of different atoms, for example  $\text{CO}^+$ , the different range of each component produces a distribution of  $dE/dx$  plotted against depth having two (or more) maxima. For the important case of molecules containing hydrogen such as  $\text{OH}^+$  the range of the proton will often be sufficiently large to cause the event to be ignored unless special precautions are taken to recognise such events.

Recent high precision experiments on the ISEE and Voyager spacecraft<sup>9-11</sup> do not seem to have sufficient range discrimination to identify a molecular ion in this way. However, the high precision with which  $dE/dx$  and  $E$  are measured in these experiments should enable these molecular ions to be recognised as a group apparently having an anomalous mass, for example,  $\text{CO}^+$  would appear to be  $^{28}\text{Ne}$  and  $\text{OH}^+$  would appear to be  $^{17,25}\text{O}!$

If fluxes of molecular ions are reasonably high, a considerable number of different species could probably be detected. Their relative abundance would give valuable insight into processes associated with the injection of material and its acceleration to produce this particular low energy component of the cosmic radiation.

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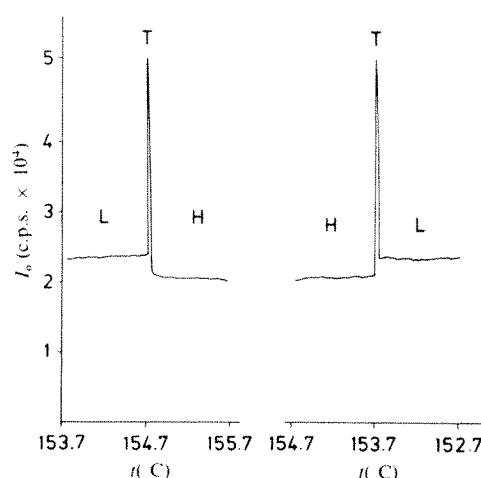
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## Increase of X-ray reflection intensities and profile widths at the low- to high- $\text{V}_3\text{O}_5$ phase transition state

An unexpected increase in peak intensity and profile half width has been observed in an X-ray diffraction experiment for many reflections from a crystal of  $\text{V}_3\text{O}_5$  kept at its phase transition temperature. One explanation of the phenomena is that the 'mosaic structure' of the crystal is altered in the phase transition state, resulting in decreased extinction and increased profile widths. The results presented here are interesting spin off products of crystallographic studies of the mixed-valency oxide  $\text{V}_3\text{O}_5$ . The semiconductor low- $\text{V}_3\text{O}_5$  transforms in a first order transition at  $155^\circ\text{C}$  into high- $\text{V}_3\text{O}_5$ , which is a 'poor metal'<sup>1</sup>. The transformation is instantaneous and reversible and implies that an ordered distribution of V(III) and V(IV) atoms is replaced by an only partially ordered distribution, the changes in the atomic positions being fairly small ( $\leq 0.1 \text{ \AA}$ ). These facts are based on the results of accurate X-ray crystal structure determinations of the two modifications at  $25^\circ\text{C}$  (ref. 2) and  $185^\circ\text{C}$  (ref. 3) respectively. We started diffraction measurements of  $\text{V}_3\text{O}_5$  at or near the phase transition temperature  $t_T$ , using the good temperature-control facility of the available non-ambient-temperature equipment<sup>4</sup>.

We found that for many strong reflections the peak intensity increased drastically when the temperature passed  $t_T$  in either direction (see Fig. 1). Note that the intensity value for any particular strong reflection usually differs very little between the two modifications. The small difference between the crystal structures manifests itself by the presence of many very weak reflections  $hkl$  with  $h+k+l=2n+1$  in low- $\text{V}_3\text{O}_5$  (space group  $P2_1/c$ ) which are all systematically absent in high- $\text{V}_3\text{O}_5$  (space group  $I2_1/c$ ). There is a general small decrease in the reflection intensities as the temperature of the crystal is increased from room temperature to just below  $t_T$  dependent on increased thermal motions of the atoms. However, the relative intensity decrease is considerably larger for reflections  $hkl$  with  $h+k+l=2n+1$  than for the rest. Therefore a (minor) continuous structural change of low- $\text{V}_3\text{O}_5$  with temperature should precede



**Fig. 1** Peak intensity of reflection 020 recorded with increasing and decreasing temperature respectively. Hysteresis effect is  $1^\circ\text{C}$ . A PAILED X-ray single crystal diffractometer (inclination geometry) was used with  $\text{MoK}\alpha$  radiation (graphite monochromator). The specimen crystal had an approximately cubic shape, the edge being about  $0.17 \text{ mm}$ . It was heated by hot nitrogen gas blown directly on to the crystal. The delivery and heating of the gas, and the temperature control were accomplished by a specially developed device<sup>4</sup>.

the discontinuous change at  $t_T$ . Thus, the difference around  $t_T$  between the two modifications of  $V_3O_5$  is probably even smaller than stated above.

The unit cell dimensions of low- $V_3O_5$  at 25 °C are<sup>5</sup>  $a = 9.859(1)$ ,  $b = 5.0416(5)$ ,  $c = 6.991(1)$  Å and  $\beta = 109.478(6)^\circ$ . The changes from low- $V_3O_5$  at 150 °C to high- $V_3O_5$  at 160 °C are<sup>6</sup>  $\Delta a = -0.13\%$ ,  $\Delta b = -0.18\%$  and  $\Delta c = 0.13\%$ . The close similarity between the unit cell dimensions of the two phases made the positions of the reflections, especially the low-angle ones, shift very little in crystal rotation angle  $\omega$  at the transition, which was important for the initial observation of the effect.

One explanation of the phenomenon is that the rearrangement disorder in the transition state reduces the degree of perfection of the crystal with decreased extinction as one result. To test this hypothesis, many reflections with severe extinction in different directions of the reciprocal space were investigated. The intensity increase at  $t_T$  was always observed, at least for  $y \leq 0.9$ ,  $y$  being the secondary extinction correction factor calculated according to Zachariasen<sup>7</sup>, and  $y = I_{\text{obs}}/I_{\text{corr}}$  where  $I$  is the integrated intensity. Table 1 presents some of the studied reflections.

Although some of the strongest reflection intensities could be expected to suffer from counting losses no corrections were

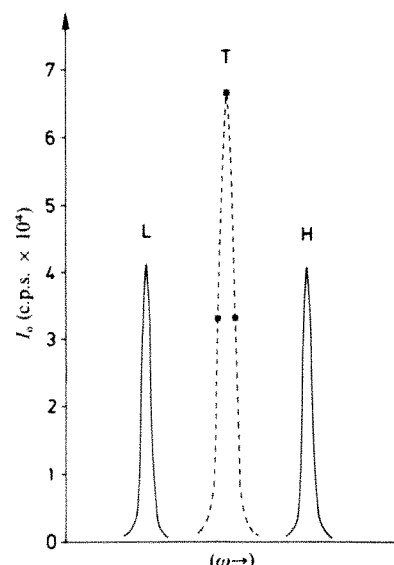


Fig. 2 Intensity profiles ( $\omega$ -scan) for reflection  $-114$  observed below (L) and above (H)  $t_T$  and constructed at (T)  $t_T$  from point observations (\*). Experimental conditions as in Fig. 1.

**Table 1** Secondary extinction correction (SEC), peak intensity and peak width: A comparison between low- $V_3O_5$ (L) at 153 °C, transition-state- $V_3O_5$ (T) and high- $V_3O_5$ (H) at 156 °C. For each reflection  $hkl$  first row means L, second T and third H

$h$	$k$	$l$	$\nu(^{\circ})$	$Y(^{\circ})$	SEC $y = \frac{I_{\text{obs}}}{I_{\text{corr}}}$	Peak intensity (c.p.s.)	Peak width ( $h$ ) at half peak intensity ( $^{\circ}$ )	Mean increase in peak width ( $\Delta h$ ), at $t_T(^{\circ})$
2	0	0	0.0	8.8		22,300	0.344	
						41,700	0.390	
					0.32	22,100	0.345	0.045
6	0	0	0.0	26.5		7,200	0.339	
						10,600	0.373	
					0.60	6,700	0.342	0.032
8	0	0	0.0	35.6		7,700	0.344	
						12,760	0.377	
					0.54	7,300	0.342	0.034
16	0	0	0.0	75.6		1,440	0.440	
						1,790	0.481	
					0.80	1,420	0.443	0.040
0	2	0	0.0	16.2		24,800	0.339	
						51,800	0.388	
					0.27	22,000	0.342	0.048
0	4	0	0.0	32.8		8,700	0.347	
						16,480	0.362	
					0.55	8,600	0.352	0.013
0	6	0	0.0	50.1		6,600	0.362	
						10,900	0.398	
					0.59	5,800	0.366	0.034
0	8	0	0.0	68.8		1,760	0.408	
						2,110	0.474	
					0.83	1,580	0.414	0.063
0	0	2	5.8	4.1		49,000	0.599	
						84,000	0.728	
					0.23	48,800	0.590	0.133
-1	1	4	11.7	9.1		41,400	0.612	
						66,900	0.878	
					0.26	41,000	0.607	0.269
-2	0	6	17.7	3.8		7,880	1.322	
						10,000	1.787	
					0.77	7,700	1.316	0.468
0	0	10	30.5	24.2		1,920	0.652	
						2,200	0.843	
					0.87	1,840	0.641	0.197

$\nu$  is the inclination angle of the detector axis,  $Y$  the angle between the detector axis and a horizontal plane containing the primary beam and the crystal rotation axis ( $//[001]$ ). The  $y$ -values actually refer to H but should be almost identical for L.

applied. However, this would mean that the real effect in these cases was even larger than the one observed.

If the crystal perfection is decreased in the phase transition state one should also observe a broadening of the width of the integrated intensity profile. Accordingly, comparative measurements of the half widths of these profiles for the reflections included in Table 1 were made just below  $t_T$ , at  $t_T$  as well as just above it. The measurements at the phase transition temperature were difficult to perform, and the time available for each of them usually was between 5 and 10 s. However, as the actual reflections had strong intensities, using the pen recorder as the intensity indicator was successful. In all cases investigated, the reflection profile more or less broadened at  $t_T$  (see Fig. 2 and Table 1). This quantity is a multiple convolution of a number of experimental components of which all but one or possibly two crystal mosaicity components, isotropy being assumed, should be fairly constant for each reflection at and around  $t_T$ . An attempt will be made to correlate the observed half width increases at  $t_T$  with the theoretical expressions for the mosaic angle distribution half width and the mosaic particle size component half width, which have both been derived by Ito<sup>8</sup>. However, a deconvolution analysis is necessary for this which is still in progress.

Similar effects of other comparable phase transitions do not seem to have been discussed previously. However, the transient intensity increase of a strong reflection at the  $\alpha$ - to  $\beta$ -quartz transition which is shown in ref. 9 may be another example.

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## Measurement of the neutron spectra from beam-heated PLT plasmas

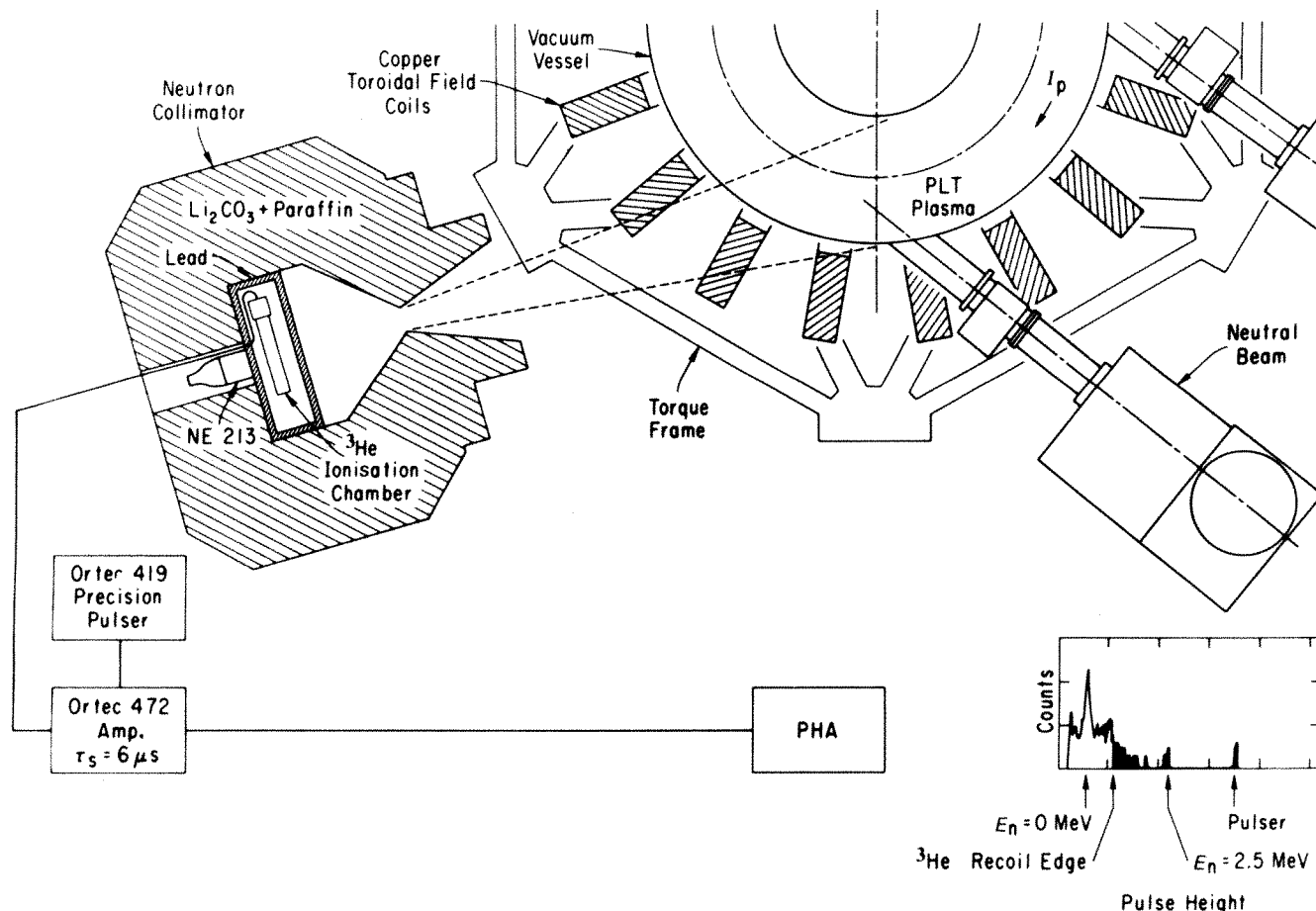
MEASUREMENTS of the neutron spectra from the PLT tokamak plasma are reported here which provide information on the centre-of-mass velocity of the reacting deuteron pairs. The observation in PLT of no significant directed velocity yields additional evidence that the neutrons are produced by a thermonuclear process. Previous spectral measurements on other fusion devices have often determined that the neutron emission was non-thermonuclear and resulted from a small 'tail' component of energetic ions<sup>1-3</sup>. The neutron spectra indicated a directed velocity of the reacting deuterons and thus non-thermonuclear neutron emission if the injected neutral beam ions were deuterium. In this condition, the neutron spectra were consistent with the expected beam-induced  $d(d,n)^3\text{He}$  reactions. However, if the heating beam used hydrogen, then the neutron spectra are consistent with a thermonuclear neutron emission resulting from the beam heating of the bulk deuterons in the plasma.

The importance of a thermonuclear neutron emission is that it allows the neutron emission measurement to be used to deduce ion temperature<sup>4</sup>. The ion temperature deduced by the magnitude of the neutron emission has been consistent with other PLT ion temperature diagnostic<sup>5,6</sup> such as charge exchange spectral observations. Although the charge exchange spectrum indicated a maxwellian ion population up to 6 T, the observation had been made only perpendicularly to the direction of the tangential neutral beam injection. Thus, we use the neutron spectral measurements to provide information on the plasma ion population in the direction parallel to the neutral beam injection where non-thermonuclear emission might be induced by the

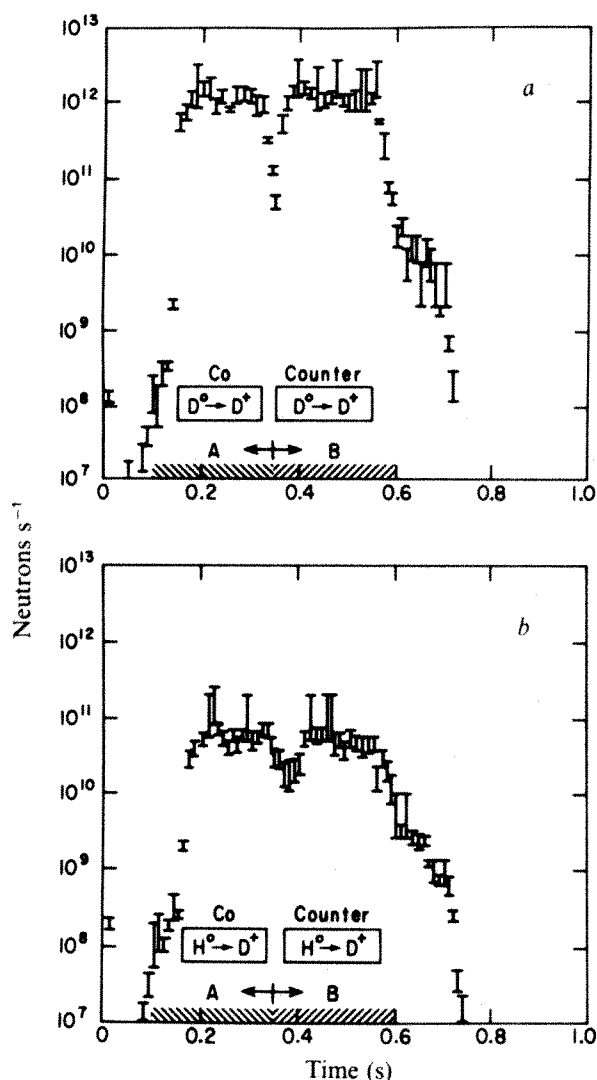
presence of trace amounts of deuterium in the hydrogen beams or by momentum transfer from the beams to the background plasma. To observe such asymmetries, a neutron spectrometer was collimated (Fig. 1) to look towards co-injected ions and comparisons were made between co-only injection and counter-only injection. In this manner, any non-thermonuclear emission induced by the beams would be observed from the shift of the mean neutron energy caused by the directed centre-of-mass velocity of the reacting deuteron pairs. Thus, the PLT neutron spectra experiment was carried out in the manner of the classic neutron spectra experiments on Zeta<sup>1</sup>. All the PLT spectra have about as many counts as in those Zeta spectra, although for PLT the spectra were obtained with time resolution and with higher energy resolution.

The PLT tokamak<sup>7</sup>, and its operation<sup>5</sup> with neutral beams have been described elsewhere. For these experiments, the plasma had a minor radius of 0.40 m as defined by carbon limiters, a major radius of 1.34 m, a toroidal magnetic field of 3.2 T, a plasma current of 0.4 MA, and titanium gettered vacuum walls. Four tangential neutral beams which can collectively deliver up to 2.5 MW of power to the plasma were used. Two of the beams (co) were directed parallel to the plasma current, and two (counter) were directed antiparallel to the plasma current.

About 200 discharges were run with hydrogen neutral beam injection into a deuterium plasma ( $\text{H}^0 \rightarrow \text{D}^+$ ) and about 15 discharges were run with deuterium neutral beam injection into a deuterium plasma ( $\text{D}^0 \rightarrow \text{D}^+$ ). In each set of discharges, the co-injectors were used early in the discharge and the counter-injectors were applied later in the same discharge after the co-injectors were turned off and the neutron emission from them had decayed. The neutron emission (Fig. 2) was about  $7 \times 10^{10} \text{ n s}^{-1}$  during the hydrogen injection ( $\text{H}^0 \rightarrow \text{D}^+$ ) and about  $10^{12} \text{ n s}^{-1}$  during the deuterium injection ( $\text{D}^0 \rightarrow \text{D}^+$ ). For the  $\text{D}^0 \rightarrow \text{D}^+$  cases, only one beam at reduced voltage was used to



**Fig. 1** The  $^3\text{He}$  ionisation chamber is surrounded by two inches of lead to reduce X-ray induced counts and is collimated by a 7-ton paraffin plus  $\text{Li}_2\text{CO}_3$  neutron collimator to view tangentially to the PLT minor axis.



**Fig. 2** The neutron emission during (a)  $D^0 \rightarrow D^+$  (about 0.3 MW in each direction) and (b)  $H^0 \rightarrow D^+$  (about 0.9 MW in each direction). The detector counting intervals were made to coincide with A, co-only injection; and B, counter-only injection. The ion temperature deduced from the magnitude of the neutron emission ( $H^0 \rightarrow D^+$ ) is about 2 keV.

keep the neutron emission low and thus prevent the neutron spectrometer from saturating.

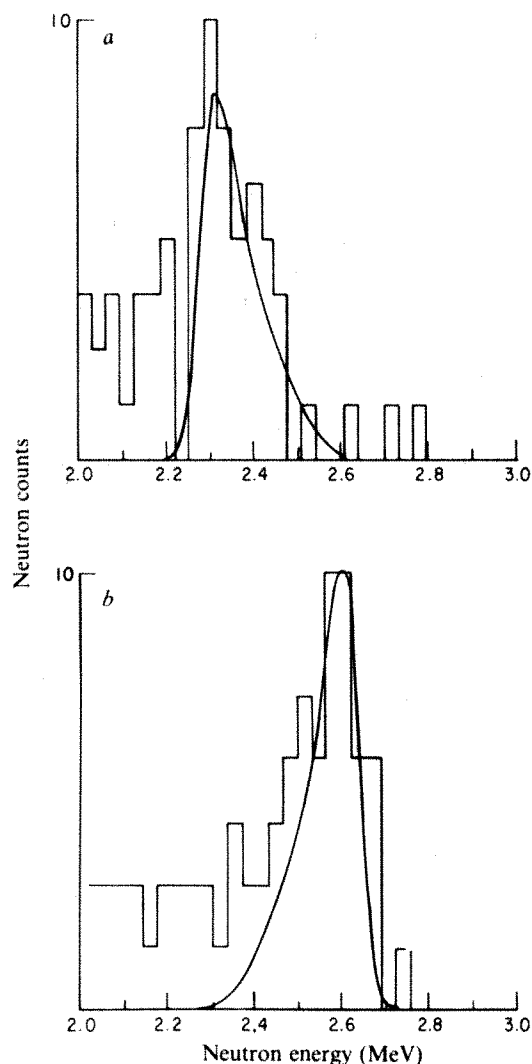
The neutron spectra were recorded by a  $^3\text{He}$  ionisation chamber (Fig. 1) and the counts were recorded separately for two different time zones during the discharge. The first time zone encompassed and recorded the co-only injection. The second time zone encompassed and recorded the counter-only injection. The early and end stages of the discharge were excluded to remove any possibility of X-ray noise induced by runaway electrons. All the discharges reported here were quite free of runaway electrons.

The neutron collimator, which is similar to one used by Glasgow *et al.*<sup>8</sup>, is composed of equal weights of paraffin and  $\text{Li}_2\text{CO}_3$  to a linear dimension of about nine absorption lengths for 2.5 MeV neutrons. The paraffin thermalises the neutrons incident on the collimator from directions other than the observation volume, and the  $^6\text{Li}$  in the natural lithium of the  $\text{Li}_2\text{CO}_3$  non-radiatively absorbs the thermal neutrons. Tests of the collimator with the observation window plugged, indicate that  $<10^{-3}$  of the total spectrometer counts occur from neutrons incident on the  $^3\text{He}$  detector from unobserved regions. The complex PLT geometry (Fig. 1) enables many neutron scattering centres to be within the line of sight, such as the massive copper coils, diagnostic equipment, and the concrete wall on the far side of PLT. These scattering centres have an unknown influence on

the recorded neutron spectra and should introduce low energy neutrons into the spectra.

The  $^3\text{He}$  ionisation chamber<sup>9,10</sup> collects the charge produced by the proton and triton in the  $^3\text{He} (n,p)t$  reaction. The detector saturates at total count rates of about  $10^4$  c.p.s., but even near saturation, only a few c.p.s. are recorded in the region near 2.5 MeV. For each  $^3\text{He} (n,p)t$  count near 2.5 MeV, there are many more  $^3\text{He}$  recoil counts, and  $^3\text{He} (n,p)t$  counts for scattered neutrons which have reached epithermal energies (spectrum in Fig. 1). The absolute energy scale was derived from the  $Q$  of the  $^3\text{He}$  reaction (0.76 MeV) and the linear response of the spectrometer. This procedure could result in the energy scale of the set of spectra being in error by  $\pm 0.05$  MeV in the region near 2.5 MeV. The spectrometer was calibrated using accelerator induced mono-energetic neutrons and has an energy resolution of about 0.055 MeV FWHM, or standard deviation of a single count. The ability of the spectrometer to determine relative mean energies should be limited only by statistics.

The  $D^0 \rightarrow D^+$  spectra (Fig. 3) indicate an energy shift of 0.26 MeV between co-only and counter-only injection, establishing the ability of the  $^3\text{He}$  detector to make noticeable neutron energy shift measurements in the PLT conditions. Each of these  $D^0 \rightarrow D^+$  spectra can be reasonably explained by the expected beam-induced reactions between the energetic injected ions and the background thermal ions. The PLT  $D^0 \rightarrow D^+$  spectra were compared to  $Z_{\text{eff}} = 5$ ,  $T_i = 1.5$  keV Monte Carlo



**Fig. 3** The measured  $D^0 \rightarrow D^+$  neutron spectra. The counter-injection voltage was 30 kV (a). The co-injection voltage was 26 kV (b).

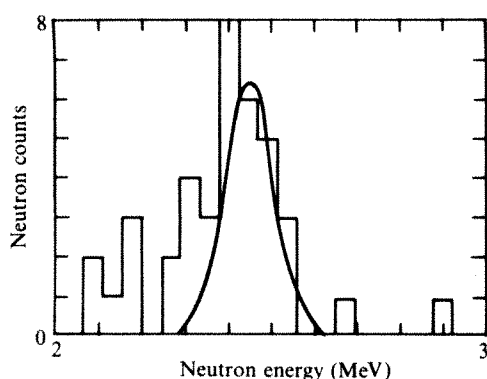


Fig. 4 The measured  $H^0 \rightarrow D^+$  neutron spectra including all the co-only and counter-only counts.

code predictions<sup>11</sup> for the collimated neutron spectra (Fig. 3). The co-injection case indicated a 60%  $\chi^2$  probability of being produced by beam-induced reactions while the counter-injection case indicated a 55%  $\chi^2$  probability of being produced by beam-induced reactions. The  $\chi^2$  test was performed only for that part of the calculated spectrum which was  $>10\%$  of the peak.

The  $H^0 \rightarrow D^+$  spectra indicate no statistically significant energy shift between the co-only and counter-only injection cases, as is expected for thermonuclear neutron emission. The predicted mean energy should be 2.45 MeV, while experimentally, the 11 co-only counts have a mean energy of  $2.41 \pm 0.02$  MeV and the 19 counter-only counts have a mean energy of  $2.42 \pm 0.01$  MeV. The downward energy shift from 2.45 MeV probably results from the energy scale calibration. The expected line width should be 0.12 MeV for the 2 keV ion temperature plasma measured by a detector with 0.055 MeV resolution. Adding all the experimental  $H^0 \rightarrow D^+$  counts together to improve the statistics (Fig. 4) results in a line width of 0.13 MeV. The observed  $H^0 \rightarrow D^+$  neutron spectrum indicated a 50%  $\chi^2$  probability of being produced by thermonuclear reactions. One degree of freedom was used in the  $\chi^2$  test to normalise the mean of the spectra and the fit was applied only for that part of the calculated spectrum  $>10\%$  of the peak.

In conclusion, no measurable centre-of-mass velocity of the reacting deuteron pairs was induced by hydrogen neutral beam injection. Furthermore, the shape of the experimental neutron emission line was consistent with a thermonuclear neutron emission at the ion temperature predicted by the magnitude of the neutron emission. These two observations are consistent with a thermonuclear origin of the PLT neutrons during hydrogen neutral beam heating of a deuterium plasma.

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## Deposition and accumulation of plutonium isotopes in Antarctica

DATA on the deposition of plutonium isotopes are presented here from the atmosphere at Dome C ( $123^{\circ}10' E$ ,  $74^{\circ}39' S$ ; 3,214 m elevation) on the high Antarctic plateau. Plutonium isotopes are among the anthropogenic chemicals that have become global contaminants<sup>1–3</sup> and it is, therefore, important to gain a historical perspective to their worldwide dispersion. The analysis of successive layers of permanent snow fields permits the determination of both present and historical fluxes of anthropogenic chemicals and other contaminants that are dispersed through the atmosphere<sup>3–6</sup>. Dome C is an ideal site for such studies: annual precipitation at Dome C is of the order of  $3.7 g H_2O cm^{-2}$  (ref. 7), considerably lower than the mean annual deposition of  $15.5 g H_2O cm^{-2}$  over the entire continent<sup>8</sup>. The mean annual temperature is  $-53.5^{\circ}C$ , with summer temperatures remaining well below freezing, precluding vertical percolation through successive layers and reducing potential losses from volatilisation.

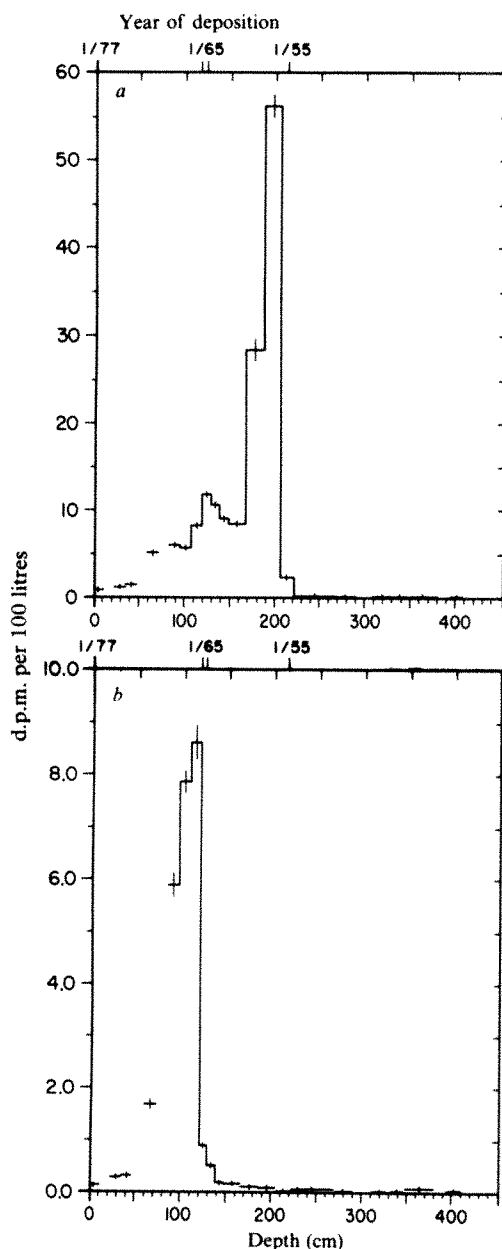
A 5 m pit was dug at Dome C in January 1977. Snow in layers of approximately 10 cm was sampled and the meltwater was transferred into 20-l, polyethylene cubitainers. They were then acidified, equilibrated, and the isotopes subsequently co-precipitated with aluminium phosphate following the technique of Koide and Bruland<sup>9</sup>. Each sample consisted of a total of 100 litres. The precipitates in the original cubitainers were returned to the US. They were dissolved in 9 M HCl and the Pu isotopes isolated following the procedures of Talvitie<sup>10</sup> and Wong.<sup>11</sup> An aliquot was removed for  $^{210}Po$  determination<sup>9</sup>. Samples were counted within 2 weeks after separation by  $\alpha$  spectrometry;  $^{208}Po$  and  $^{242}Pu$  were used as yield tracers.

Figure 1 shows the activities of  $^{239+240}Pu$  and  $^{238}Pu$  versus depth of snow. Assignment of the 1955 time horizon to 214 cm, at the beginning of the rise in  $^{239}Pu$  activity, was based on previous work at Dome C by Lorius<sup>7</sup> and studies undertaken elsewhere in Antarctica<sup>12–14</sup> which have shown a sharp increase in  $^{90}Sr$  and gross  $\beta$  activity in strata deposited in early 1955. This assignment is consistent with our  $^{210}Pb$  results. Assuming constant sedimentation for the depth interval, 0–250 cm,  $^{210}Pb$  activity at any depth  $z$ ,  $A_z$ , can be expressed by  $A_z = A_0 \exp(-\beta z)$ . Our data yielded an  $A_0$  of 142 d.p.m. per 100 litres and a  $\beta$  of  $-0.00308 cm^{-1}$ , where  $r = 0.90$  for the best-fit curve. Thus, a time interval of  $21.2 \pm 2.4 yr (1\sigma)$  to this 214 cm depth was calculated. Our assignment of the 1965 time horizon interval was derived by interpolation and estimates of maximum error due to compaction<sup>15</sup>.

The profile of  $^{239+240}Pu$  deposition (Fig. 1a) is unlike the profile observed in Greenland<sup>7</sup> in that the majority of the total fallout occurred before 1960, whereas in Greenland the greater portion of the deposition occurred after 1960. Significant quantities of  $^{239+240}Pu$  began to appear in the snow layer corresponding to the estimated date of 1955. The ensuing peak's size and timing suggest that a significant portion of the  $^{239+240}Pu$  could have been contributed by the US Castle test, Bravo, on 28 February 1954, at Bikini ( $165^{\circ} E$ ,  $11^{\circ} N$ ). This 15 megatonne detonation derived a major portion of its energy from the



fusion-induced fission of  $^{238}\text{U}$  (ref. 16). Large quantities of  $^{239}\text{Pu}$  were probably produced from  $^{238}\text{U}$  by neutron capture. Previous studies<sup>12-14</sup> have shown that significant stratospheric fallout from this test began to arrive in the Antarctic  $12 \pm 2$  months later. Further weapons tests at Bikini and Eniwetok such as the Hardtack series during 1958 might have contributed additional  $^{239+240}\text{Pu}$  to this major peak.  $^{90}\text{Sr}$  attributed to these tests has been observed at several Antarctic sites with an arrival date of early 1959<sup>13</sup>. The smaller amounts of  $^{239+240}\text{Pu}$  deposited at Dome C after 1960 suggest that the tests conducted in the early 1960s, primarily in the Northern Hemisphere, resulted in a relatively low level of stratospheric transfer to the high latitudes of the Southern Hemisphere.



**Fig. 1**  $^{239+240}\text{Pu}$  (a) and  $^{238}\text{Pu}$  (b) activities at Dome C, Antarctica. Horizontal lines depict the depth of each layer sampled. Vertical lines within each sample represent the uncertainty due to counting statistics ( $1\sigma$ ). Plutonium sample counting times ranged from 2 to 9 d. The minimum detectable amount of  $^{238}\text{Pu}$  and  $^{239+240}\text{Pu}$  was found to be of the order of 0.03 d.p.m. per 100 litres. Samples for the depth intervals, 11.9–23.9 cm, 48.0–59.9 cm, and 71.9–83.8 cm were not obtained.

Figure 1b shows the snow record of  $^{238}\text{Pu}$  at Dome C. The April 1964 burnup of a satellite (SNAP-9A) containing 17 kCi of  $^{238}\text{Pu}$  in the Southern stratosphere<sup>1</sup> is recorded as a rapid activity increase in the 1965–66 stratum. The deposition of  $^{238}\text{Pu}$  closely parallels the changes in the stratospheric inventory of SNAP  $^{238}\text{Pu}$  over the period 1964–69 as shown by Harley<sup>17</sup>. The activity ratio of  $^{238}\text{Pu}/^{239+240}\text{Pu}$  also rises sharply, reaching a maximum of 150% in the layer assigned to approximately 1967 (the stratum following the peak activity). Harley<sup>17</sup> reported comparable high activity ratios in Southern Hemisphere surface air samples in 1967 and 1968.

During the period of peak fallout of  $^{239+240}\text{Pu}$  (1955–59), levels of  $^{238}\text{Pu}$  activity were markedly low ( $^{238}\text{Pu}$  values of  $0.10 \pm 0.02$  and  $0.12 \pm 0.03$  d.p.m. per 100 litres compared to the respective  $^{239+240}\text{Pu}$  values of  $56.2 \pm 1.3$  and  $28.4 \pm 1.2$  d.p.m. per 100 litres). Harley<sup>17</sup> suggested that a  $^{238}\text{Pu}/^{239+240}\text{Pu}$  ratio of 3% might be representative of weapons fallout. If this was the case, then a significant  $^{238}\text{Pu}$  peak should have been associated with the maximum fallout of  $^{239+240}\text{Pu}$ . However, this was not observed, and in fact, the activity ratio was of the order of 0.3%, an order of magnitude lower. Harley<sup>17</sup> pointed out that the value of 3% was not well established since good  $\alpha$  spectrometry was not available on their samples before SNAP-9A. Thus, this ratio may be doubtful as a widely applicable value. This discrepancy warrants further investigation.

To calculate the fluxes of  $^{239+240}\text{Pu}$  and of  $^{238}\text{Pu}$ , a mean density of  $0.352 \text{ g cm}^{-3}$ , derived from the data of Lorius<sup>7</sup> was used. The flux of  $^{239+240}\text{Pu}$  was calculated by integrating over the depth interval 0–222.5 cm. This value was found to be  $90.4 \text{ d.p.m. m}^{-2}$ , while the peak of  $19.2 \text{ d.p.m. m}^{-2} \text{ yr}^{-1}$  occurred during 1956–57. Similarly, after decay correction the flux of  $^{238}\text{Pu}$  was determined to be  $14.4 \text{ d.p.m. m}^{-2}$ . The peak flux of  $3.22 \text{ d.p.m. m}^{-2} \text{ yr}^{-1}$  occurred around 1965–66. Hardy *et al.*<sup>1</sup> using soil data have estimated cumulative worldwide fallout of plutonium isotopes as a function of latitude. Their estimates for  $70\text{--}80^\circ \text{S}$ , based on extrapolation from Southern Hemisphere samples, were  $0.03 \pm 0.01 \text{ mCi km}^{-2}$  of  $^{239+240}\text{Pu}$  and  $0.008 \pm 0.005 \text{ mCi km}^{-2}$  of  $^{238}\text{Pu}$  derived from SNAP-9A. Our results provide corresponding values of 0.04 and 0.006, respectively, in good agreement with the estimates cited above.

The profiles of Fig. 1 suggest that almost all of the plutonium isotopes injected into the atmosphere have now been removed. The activities of both  $^{239+240}\text{Pu}$  and  $^{238}\text{Pu}$  deposited during 1976 at Dome C were only 1.4% of the activities deposited during the respective periods of maximum fallout.

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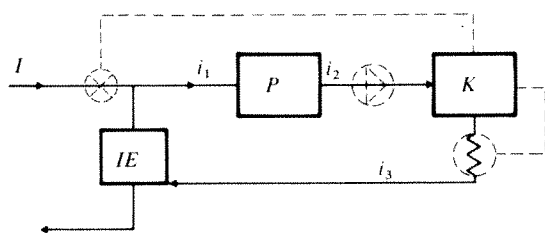
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## Climate and thermodynamic systems of maximum dissipation

THE Earth-atmosphere is a classic example of a closed, dissipative and nonlinear thermodynamic system which is subject to both regular and irregular impulses causing significant departure from steady state. It is closed because it exchanges energy (solar and thermal radiant energy) but not mass with its environment. It is dissipative because the net input of radiant energy occurs mainly in regions of high temperature towards the Equator and the net output occurs mainly in regions of low temperature towards the poles. It is nonlinear basically because of the multiplicity of internal feedbacks and because of the importance of advective processes. It has steady-state character in the sense that the annual mean radiant energy input is very close to the annual mean output, and parameters such as the annual mean temperature do not vary significantly from one period to another. The regular seasonal variation in solar position ensures significant departure from the steady state so defined, and there are also significant irregular departures arising (for instance) from variations of solar input and IR output caused by variations in the amount and distribution of cloud. Recently I have shown<sup>1</sup> that the overall Earth-atmosphere climate system seems to have adopted a format whereby the total thermodynamic dissipation associated with the horizontal energy flows in the atmosphere and ocean is a maximum. 'Format' in this context refers to the annual average geographic distribution of cloud, surface temperature and the horizontal energy flows. The practical significance of this is that, if one could accept it as a general principle governing climate behaviour, one could use it directly as a means of *a priori* prediction of climate and climate change without needing detailed analysis of the internal workings of the system. I could not explain previously why the Earth-atmosphere system should be so constrained. This note points out that the Earth-atmosphere has characteristics such that it might be expected to obey such a constraint. Furthermore, these characteristics are sufficiently general that the same principle of selection of steady-state mode of maximum dissipation may apply to a broad class of non-linear systems.

A fair thermodynamic representation of the global climate system is as depicted by the solid lines in Fig. 1. There is an input of solar energy  $I$ , most of which is converted directly into various forms of internal energy  $IE$  and is returned to space in the form of thermal IR radiation. Some of the input appears as available potential energy  $P$ , is converted to kinetic energy  $K$ , and is in turn dissipated by friction into internal energy before being radiated back to space. At steady state the rates of creation of



**Fig. 1** The total Earth-atmosphere system of: energy transfers (thin solid lines); reservoirs of internal, available potential, and kinetic energy ( $IE$ ,  $P$  and  $K$  respectively); feedback controls from kinetic energy (dashed lines); and an indication of the non-linearity of the rate of potential energy conversion (dashed diode symbol). Adequate data are not yet available for the oceanic part of the total system, but for the atmospheric component the annual average quasi-steady state values are:  $i_1 = i_2 = i_3 \approx 2.3 \text{ W m}^{-2}$ ;  $K \approx 1.5 \times 10^6 \text{ J m}^{-2}$  with the ratio  $P:K \approx 10:1$  (refs 2, 7).

available potential energy, conversion of available potential to kinetic energy and the dissipation of kinetic energy ( $i_1$ ,  $i_2$  and  $i_3$  respectively) are equal.

The dashed components of Fig. 1 concern two characteristics of importance to the present argument. First, there are feedbacks such that both the rate of energy input ( $I$  or  $i_1$ ) and the rate of dissipation  $i_3$  are functions of, or can in principle be related to, the total kinetic energy. (One of the prime controls on the rate of energy input is, for instance, cloud cover; and cloud cover is a complex function of the amount and distribution of the large and small-scale motions of the atmosphere and ocean, of quantities which determine the motion, or of quantities which are determined by the motion.) Second, the rate of conversion  $i_2$  of potential to kinetic energy is a highly non-linear and increasing function of potential energy. Some examinations of the process effectively envisage an  $i_2 = (P - P_c)^n$  relation where  $P_c$  is some critical value of potential energy below which the rate of conversion is very small<sup>4</sup> and  $n > 1$ .

Let  $i_1$  and  $i_3$  be functions of kinetic energy as in Fig. 2. The actual shape of the curves is irrelevant. The important characteristics are: (1) There is the possibility of a number (and in the case of the Earth-atmosphere system, a large number) of steady state positions where the rate of energy input equals the rate of output (that is  $i_1 = i_3$ ); and (2) the dissipation rate  $i_3$  is an increasing function of kinetic energy.

The real system is sufficiently complex in its detailed interactions for there to be considerable natural variability in, for instance, cloud cover and the rate of energy input. Therefore there is variability about whatever is the steady state determined by the current average value of  $K$ . The extent and character of this variability is unknown even for the quasi-steady state which the Earth-atmosphere system presently occupies. However, consider at some stage that the system was in one of the mid-range stable regimes (A of Fig. 2 say). The input  $I$  or  $i_1$  will be subject to natural variability which may or may not be random and is not necessarily a function of  $K$ , but may occasionally be large enough to boost the system to another stable regime. If the variability is random, presumably an impulse of  $+\Delta Q$  in  $i_1$  is as likely to occur as an impulse of  $-\Delta Q$ , and in the absence of other information the system is as likely to change to a new steady state of lower average  $K$  as to one of higher average  $K$ .

However, the non-linearity of the conversion of  $P$  to  $K$  ensures that for large values of  $\Delta Q$  (short term changes of  $i_1$  away from its steady state value where they are not necessarily an immediate function of  $K$ )

$$\frac{\Delta K^+}{\Delta Q} > \frac{\Delta K^-}{-\Delta Q} \quad (1)$$

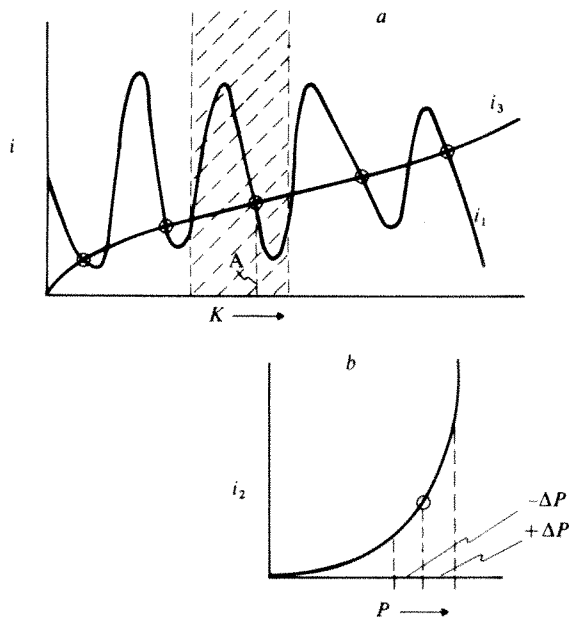
That is, a positive impulse  $\Delta Q$  in  $i_1$  is more likely to change the system to a steady state of higher average  $K$  (and dissipation) than is a negative impulse in  $i_1$  likely to change the system to a steady state of lower average  $K$ . This is obvious (see legend to Fig. 2) but is not easy to prove in a rigorous manner. A complete analysis would require a fairly sophisticated application of control theory. (It would require knowledge of the exact form of the random (?) impulses, and some specific knowledge of at least the statistical distribution of steady states as a function of  $K$ . Such information is not yet available for the Earth-atmosphere system.) Suffice it to say that first order treatment leads to the relationship

$$\frac{\Delta K^+}{\Delta Q} - \frac{\Delta K^-}{-\Delta Q} \approx \frac{b\epsilon}{1 + 2cZ + c\epsilon + c^2 + c^2Z^2 + c^2Z\epsilon} \quad (2)$$

where, if  $\Delta t$  is the short time period over which the impulse is applied and  $a$  is the average slope of the  $i_3$  versus  $K$  relationship and  $\Delta P$  or  $-\Delta P$  are the net responses in available potential energy to  $\Delta Q$  or  $-\Delta Q$  respectively, then

$$b = \frac{1}{2}\Delta t / (1 + \frac{1}{2}a\Delta t); \quad c = \frac{1}{2}\Delta t; \quad Z = \Delta i_2 / (-\Delta P);$$

and  $\epsilon = \Delta i_2 / \Delta P - \Delta i_2 / (-\Delta P)$



**Fig. 2** *a*, The functions relating average rate of energy input  $i_1$  and the rate of dissipation  $i_3$  to the average total kinetic energy  $K$ . The circled points are possible stable steady states. The uncircled points where  $i_1 = i_3$  are unstable steady states because of the positive feedback associated with slight displacements of  $K$ . The hatched area roughly indicates the extent of the stable regime about one such steady state  $A$  (that is between the adjacent unstable steady states). The central question is why short-term random variability of  $i_1$  about the average value determined by  $K$  should (if the impulses are large enough) preferentially boost the system to steady states of higher average  $K$  and ultimately to maximum  $i_3$  or dissipation. The basic reason for the system under discussion is super-linearity in the rate of conversion  $i_2$  of available potential energy  $P$  (see *b*). Sub-linearity would tend to ensure movement to steady states of lower average  $K$ . The intuitive argument (see text) is as follows:  $+\Delta Q$  on  $i_1 \rightarrow$  growth of  $P \rightarrow$  much increased  $i_2 \rightarrow$  large growth of  $K$ .  $-\Delta Q$  on  $i_1 \rightarrow$  decay of  $P \rightarrow$  slightly decreased  $i_2 \rightarrow$  slight decay of  $K$ .

Note that the changes  $\Delta i_2$  in  $Z$  and  $\varepsilon$  refer to the result of changes  $\Delta P$  and  $-\Delta P$  away from the steady state value of  $P$ . Thus, if one accepts  $\Delta K^+ / \Delta Q - \Delta K^- / (-\Delta Q)$  as a measure of the 'strength'  $S$  of the tendency under random forcing to move to steady states of higher dissipation, then large  $S$  is ensured among other things by greater positive non-linearity  $\varepsilon$  in the rate of conversion of potential to kinetic energy. It is ensured also by smaller slope of the  $i_3$  versus  $K$  relation.

Provided (1) The rate of conversion of  $P$  to  $K$  is a super-linear increasing function of  $P$ ; (2) the rate of dissipation is an increasing function of  $K$ ; (3) the system has several possible stable steady states; and (4) there is random variability in the rate of energy input which is, or was at some time, of sufficient magnitude to boost the system from one steady state to another, then it can be expected that the system will ultimately occupy that steady-state regime which has maximum dissipation. As far as the Earth-atmosphere is concerned all the conditions are acceptable, but are difficult to prove absolutely. In particular, it has yet to be proved that the dynamical constraints on atmospheric and oceanic motions allow the existence of more than one of the steady states otherwise allowable on energy balance considerations alone. Furthermore, the possibility must be considered that the distribution of possible steady states as a function of average kinetic energy is so extraordinary that the mechanism envisaged here is effectively negated.

Note that 'random variability of input' can be occasioned by processes internal to the system. It is not necessary to assume changes in some external parameter such as the solar constant.

In general terms the above limitations are sufficiently broad and non-restrictive to suggest that the overall concept may be applicable to a large class of non-linear thermodynamic systems

where there is 'series' conversion of energy from one form to another. In the Earth-atmosphere example, the non-linearity of the conversion of  $P$  to  $K$  is particularly obvious ( $\varepsilon$  of equation (2) is large) and presumably once the system is in its ultimate format there is no tendency to return to a regime of lower dissipation. However, one can imagine systems where the non-linearity is weak and the ultimate behaviour would be no more than a tendency to spend more time in regimes of higher dissipation. Similarly a system of strongly sub-linear energy conversion (negative  $\varepsilon$ ) would be expected to move towards the stable steady state of minimum dissipation.

The Earth-atmosphere example effectively involves a 'series' system of energy conversions, and the state of maximum dissipation is also the state of maximum rate of energy transfer—a condition which has been the focus of several investigations in this and other branches of fluid mechanics<sup>5,6</sup>.

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## Is there any scientific explanation of the paranormal?

THE apparent impossibility of the occurrence of 'paranormal' phenomena has not discouraged their extensive investigation, although there has not been any uniformly accepted validation or explanation by the scientific community. To clarify exactly how difficult ESP phenomena are to explain, it is necessary to place them in the framework of modern science. Explanations of the phenomena have been brought forward which have been claimed to make them more respectable. These explanations must also be looked at from the point of view of modern science and this paper is devoted to that task. In particular we wish to indicate that on theoretical grounds the only scientifically feasible explanation could be electromagnetism (EM) involving suitably strong EM fields. Thus we regard that this paper completes our earlier work<sup>1</sup> where we presented experimental results giving the level of the EM signals emitted by subjects when engaged in supposedly paranormal activity. These EM levels were many orders of magnitude lower than the ones we calculate here as needed to achieve paranormal effects. Taken together the two papers are a strong argument against the validity of the paranormal.

We will start our analysis by applying a criterion to which psychic phenomena and its explanations must conform, that of the conservation of energy (stemming as it does from the underlying symmetry of the forces of nature).

From energy conservation we may roughly assess the amount of energy transfer required to achieve a particular paranormal effect and hence assess its feasibility. The energies associated with reincarnation and precognition cannot be assessed at all; that for materialisation is expected to be of the order of megajoules if reasonably based on the energy required to break the molecular bonds in one mole of a normal material (500 kJ mol<sup>-1</sup>). The energies needed for psychokinesis (PK), spoon-bending and poltergeists is of the order of joules, assuming that the phenomena occur by the gross effects to which they correspond, this being the movement or deformation of macroscopic objects. Finally the phenomena of dowsing, telepathy, distant-viewing, clairvoyance and faith-healing should need only of the orders of millijoules if it is assumed that they



require activation of certain portions of the brain of the subjects involved, though far more energy may need to be emitted by the source of these effects (as in telepathy or psychic healing). Only these latter phenomena can therefore be regarded as feasible, those requiring joules of energy being unlikely though faintly possible, whilst the first three phenomena contradict modern physics.

There are a range of natural energy sources which could be conjectured as being utilised to achieve so-called paranormal effects. Cosmic rays<sup>2</sup>, nuclear beams<sup>3</sup>, neutrinos<sup>3</sup>, gravitons<sup>4,5</sup> or other controls of gravity<sup>4,5</sup> have all been suggested, though they all have such low power levels, survival value or high energy (by factors of about  $10^{20}$ ) required for control as to be completely irrelevant. A fifth force of nature<sup>6,7</sup> explaining solely ESP phenomena would be purely *ad hoc*; faster-than-light particles<sup>8</sup> and advanced potentials<sup>9</sup> invoked to explain precognition have either severe theoretical difficulties<sup>10</sup> or failed to appear<sup>11-14</sup>. Higher dimensions for materialisation and precognition<sup>15,16</sup> would be *ad hoc* as well as destroying the renormalisation program in quantum field theory.

None of the previous seven approaches suggest any mechanism by which human activity can bring about the desired paranormal phenomena. This is obviously not the case with theories in which the mind is independent of the body to some degree, such as those of Eccles<sup>17,18</sup> and Popper<sup>19,20</sup>, whose papers appear to contain no falsifiable statements.

Numerous attempts have been made to use the difficulties associated with measurements in quantum mechanics to explain paranormal phenomena<sup>21-23</sup>. However, there seems to be considerable confusion in the motivation for these attempts, especially as the standard statistical interpretation of quantum mechanics<sup>24,25</sup> has none of the claimed paradoxes. For paranormal effects to be explained in such a way they must contradict traditional quantum mechanics at some point, for which there is no other evidence.

From the viewpoint of modern science, the most reasonable approach to explain ESP, is electromagnetism (EM). We have previously reported<sup>1</sup> an extensive programme both of detection of EM radiation from subjects over nearly the whole spectrum and human body sensitivity to low-level EM radiation over a restricted, though very relevant, frequency range. We concluded that, for the subjects investigated and the phenomena associated with them (metal-bending, dowsing, psychic healing, telepathy and distant-viewing) EM cannot explain the effects. All the successful cases of PK<sup>1</sup> turned out to be completely explicable either by electrostatics or heat; we will not consider them here.

We shall now detail how difficult, if not impossible, it is for EM to account for ESP phenomena other than PK in the above-mentioned ways.

The human body is nearly a black body of temperature of 310 K that otherwise emits non-black-body EM radiation over a restricted range of physiological frequencies (d.c. to 5–6 kHz). As part of its black-body activity, the body radiates heat, mainly in the near infrared frequencies at wavelengths of  $\sim 10\ \mu\text{m}$ , the power level being  $\sim 10\ \text{mW cm}^{-2}$ . When investigating the problem of efficient emission of non-ionising EM radiation by the body in order to cause ESP effects of some kind, two crucial features have to be analysed: (1) spatial resolution, and (2) penetration depth into the body (or the emission from inside the body). Although the penetration depth inside the body increases as the frequency of the radiation decreases<sup>26</sup>, if one allows the wavelength to increase beyond a certain limit the spatial resolution becomes very poor. This may not be crucial if it is just a question of emitting energy in all directions and so hopefully producing some paranormal effect in an arbitrary direction. It is critical if small objects are to be viewed by the emitted energy, such as in dowsing for a stream a metre or so wide, or clairvoyantly attempting to discern a picture a few centimetres wide on the face of a card.

We need only consider the attempt to use EM radiation for ESP purposes from the body or elsewhere at frequencies below 10 GHz. Above that frequency the penetration depth inside the

body is practically zero<sup>26,27</sup> and EM emission will be determined completely by surface temperature. Small changes of the latter by several degrees have been reported to be possible to achieve by practice. These can produce at most only a 1% change of the black-body emission from the human body and so will be useless to explain any of the ESP phenomena requiring joules of energy mentioned earlier. If we think of dowsing, telepathy or distant-viewing as produced by an active or passive radar method using the infrared emission of the body, suitably pulsed (if that were possible), the reflected or received signal would be many orders of magnitude below the known sensitivity of the skin to infrared, such as in cases of dowsing and clairvoyance cited above and others typical in the ESP literature where positive results have been claimed. Temperature changes, both of healer and patient, do occur in the faith healing situation, but do not seem to be related to any healing process<sup>1</sup>. Thus we conclude that skin emission or sensitivity cannot be relevant to ESP, and turn to frequencies below 10 GHz for which EM penetrates the human body appreciably.

For EM of radio frequencies lower than 100 MHz spatial resolution is poor due to a wavelength  $\lambda > 3\ \text{m}$ . This seems to rule out dowsing, distant-viewing and clairvoyance, due to inability to perceive objects smaller than 3 m across by such radiation. Dowers might still detect very small static magnetic fields, and underground objects can be associated with local magnetic field anomalies of the order of  $10^{-5}\ \text{G}$ . Our results<sup>1</sup> showed that dowers were unable to detect the presence of a magnet of  $\sim 200\ \text{G}$ . Therefore, it seems very unlikely that this mechanism is involved. At the lower end of the range, energy radiation is not a suitable concept, and static field ideas are more appropriate, especially in the non-black-body region below 5 kHz. Tests with healers<sup>1</sup> have indicated no changes in such levels to within millivolts of skin potential during healing sessions. Thus we can exclude this frequency range for faith healing also. Telepathy may be conjectured to occur by ELF/VLF<sup>28,29,30</sup>, especially as there is the well known Schumann resonance at 8 Hz which allows for megametre propagation with little attenuation in the Earth-ionosphere cavity. However, the coupling of the human body to the long-wavelength radiation is especially crucial (a factor (human height/ $\lambda$ )<sup>2</sup>  $\sim 10^{-14}$  entering here). Kogan's analysis<sup>30</sup> does claim that somewhat higher frequencies are just feasible. However, values for the biocurrents induced in the receiver and required for the successful telepathic transmission of information are at most  $10^{-12}\ \text{A}$  for a distance of a few metres between sender and receiver; for a 1-km separation this is reduced to  $10^{-18}\ \text{A}$ . The possible values of biocurrents produced by activity of a group of nerve cells is about  $10^{-6}\ \text{A}$ , so the induced currents are at least  $10^6$  ( $10^{12}$  for telepathy over 1 km) below that expected to noticeably bring a change of activity in a group of nerve cells. Even a single nerve cell, requiring at least  $10^{-9}\ \text{A}$  to activate it, would have too low a current induced by such telepathic signals by a factor of  $10^3$ ; as at least  $10^3$  such cells would need to be activated to 'notice' the signal, the final factor of  $10^6$  is appropriate.

The more energetic paranormal phenomena, such as movements occurring in poltergeist activity involving heavy objects, are also readily ruled out, specifically due to the energy transfer needed. For metal bending, low-frequency fields above the breakdown strength of air would be required to produce bending or breaking in metal objects. For example, for a typical strip of metal with EM frequency of 10 kHz, power absorption by the metal of 1 mW would require an electric field of  $10^6\ \text{V m}^{-1}$ .

We finally turn to the more restricted frequency range of 100 MHz to 10 GHz. There is strong evidence<sup>27</sup> against non-thermal emission of radiation by the human body or even non-thermal effects<sup>31</sup> induced in the body by very low levels of EM radiation. The power level emitted by the human body at microwave frequencies of 1–5 GHz is of the order of  $10^{-14}\ \text{W}$ . Therefore we can immediately discard the EM explanation for the more highly energetic phenomena that is materialisation, poltergeist, metal-bending and PK (translational motion of

objects). Clearly these are too low in energy by a factor of  $10^{12}$  at least and up to  $10^{20}$  for materialisation. One of the less energetic phenomena, psychic healing, would be also down by a factor of  $10^{11}$ . It only remains to be seen how EM stands as regards the other less energetic phenomena, such as dowsing, telepathy, clairvoyance and distant-viewing. Two types of mechanisms can be envisaged for the less energetic phenomena: an active or a passive radar-like type.

Consider first site-dowsing. If we think of a dowser being able to detect underground water by means of an active radar-like mechanism, we see that radiation in the range 1–5 GHz at a power level of  $10^{-14}$  W would be attenuated when penetrating through the soil, its skin depth being of a few cm. If a dowser used a passive radar-like mechanism, this would involve him sensing a cold spot (the underground stream) from an otherwise warmer environment at a depth, say, of 20 m. Our tests<sup>1</sup> on human sensitivity to low-level (1–5 mW) EM radiation in this frequency range indicate a lack of sensitivity by a factor of  $10^{10}$ . The problems are even more difficult for map-dowsing because of the distance sometimes involved. The active radar mechanism can be ruled out, as the propagation distance of EM radiation of frequencies of 1–5 GHz is  $<100$  km and dowsers have claimed map-dowsing successes up to a few thousand kilometres. The upper limit for round-the-world propagation of an EM signal is 70 MHz ( $\lambda \geq 40$  m)<sup>32</sup>, and even then at power levels at least  $10^{14}$  times greater than those from humans. There is also the problem of spatial resolution, as  $\lambda \geq 40$  m and it could never account for the size of objects perceived (a few centimetres in some cases).

The cases of clairvoyance (direct or remote) and distant-viewing<sup>33</sup> also involve the problem of the size of the object perceived as compared with the wavelength that could be used by the subject ( $\lambda \sim 6$ –300 cm). The minimum size  $d_{\min}$  of an object observable at a distance  $R$  from an aerial of the size of the human body using radiation limited to a maximum frequency of 3 GHz for deep body penetration, satisfies  $d_{\min} \geq R/40$ , from standard diffraction theory. If  $R \sim 1$  m,  $d_{\min} \geq 2.5$  cm, contradicting the supposed ability in ref. 34 to read symbols down to 1/30 cm in size on a card a metre away. If  $R \sim 4$  km,  $d_{\min} \geq 100$  m, contradicting the distant-viewing abilities reported in the SRI tests<sup>33</sup> of observations of objects only 1 cm across.

Telepathy involves one subject (sender) transmitting encoded information to another. The receiver must then sense the incoming information, decode it and produce an output that can be compared with the original one sent to him. Here again, it is in the frequency range 1–5 GHz that this transmission would be most efficient. However, if we assume that the sender is using black-body EM radiation he is emitting in that frequency range, (necessarily above 10 kHz<sup>27</sup>), the power level of the signal would be so low (by a factor of  $10^5$  for people 1 km apart) that it could never be sensed by the receiver. Our experiments have confirmed this by showing that human sensitivity is very poor at these frequencies<sup>1</sup>.

We therefore conclude that neither EM nor any other scientific theory can explain any of the above mentioned ESP phenomena.

In particular there is no reason to support the common claim that there still may be some scientific explanation which has as yet been undiscovered. The successful reductionist approach of science rules out such a possibility except by utilisation of energies impossible to be available to the human body by a factor of billions.

We can only conclude that the existence of any of the psychic phenomena we have considered is very doubtful.

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## A pachycephalosaurid dinosaur from Madagascar and a Laurasia–Gondwanaland connection in the Cretaceous

THE discovery of a pachycephalosaurid dinosaur in the Upper Cretaceous of Madagascar is reported here. This finding casts new light on the existence of a land connection (or several such connections) between Laurasia and Gondwanaland sometime in the Lower or Mid-Cretaceous. Hitherto this family of dome-headed ornithischians had only been known from the Northern Hemisphere: from the Lower Cretaceous of England<sup>1</sup> and the Upper Cretaceous of North America<sup>2</sup> and East Asia<sup>3</sup>.

Previously the evidence for a faunal connection between Laurasia and Gondwanaland during the Cretaceous was slight<sup>4</sup>. The frequently cited cosmopolitan distribution of the sauropod dinosaur *Titanosaurus* is based on very incomplete and often plainly non-diagnostic material and it is not improbable that several related genera are involved. The discovery of a dromaeosaurid in the Upper Cretaceous of Brazil has been reported<sup>4</sup> but the lack of detailed information or illustrations makes evaluation of this record impossible. Hadrosaurine hadrosaurs are now known from several specimens from the Upper Cretaceous of Argentina<sup>5,6</sup>; they previously represented the only, and more substantial, evidence for faunal exchange between Laurasia and Gondwanaland sometime in the Cretaceous. Fragmentary tyrannosaurid remains have been described from the Upper Cretaceous of India<sup>7</sup> and South America<sup>8,9</sup>. All other records of Laurasian dinosaur families from the Southern Hemisphere, which evolved solely within the Cretaceous period, are based on extremely fragmentary material which either lacks the diagnostic characters of the respective group or has been incorrectly determined<sup>7</sup>.

The supposed presence of a ratite bird, a member of a group otherwise restricted to the Southern Hemisphere, in the Upper Cretaceous of Mongolia is very doubtful; this form has been described on the basis of two badly crushed and incomplete



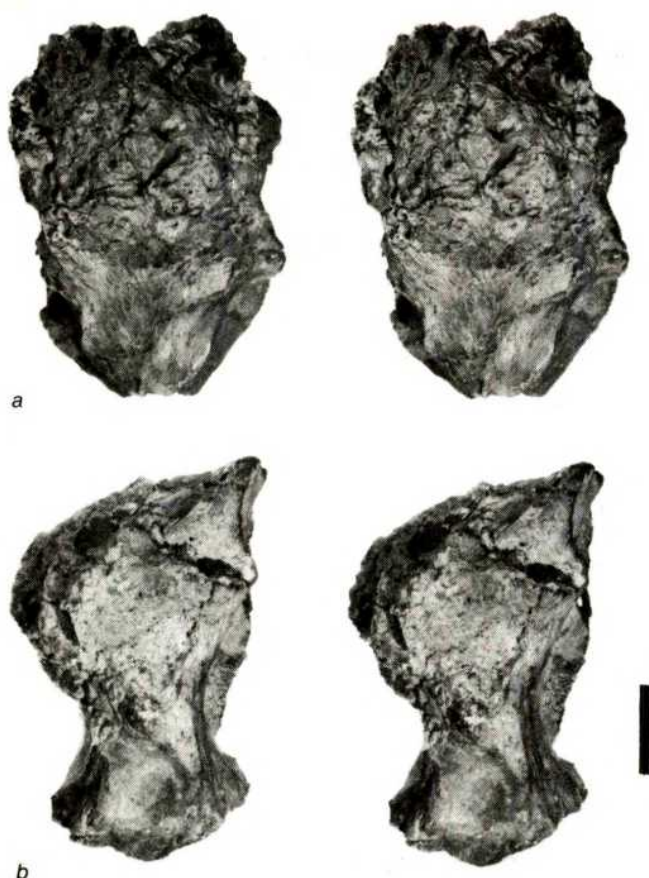


Fig. 1 *Majungatholus atopus*, new genus and species. MNHN.MAJ 4. Partial skull; stereo-photographs in dorsal (a) and lateral (b) view. Scale bar, 3 cm.

skulls<sup>10</sup>. It is quite unlike any known palaeognathous bird<sup>10</sup> and in our opinion even its avian nature is not well established<sup>9</sup>.

The new find of a pachycephalosaurid comes from the Upper Cretaceous (probably Campanian) of the Majunga District in northwestern Madagascar. It was recently discovered by P.T. The specimen was collected at the beginning of this century; its true nature was not recognised at that time. The deposits in the Majunga region have yielded a rather diversified fauna of vertebrates<sup>9,11,12</sup>, including a sauropod, a theropod and two teeth first referred to *Stegosaurus*<sup>13</sup> but probably referable to some other group of ornithischians<sup>9</sup>.

Class: Reptilia

Order: Ornithischia

Suborder: Ornithopoda

Family: Pachycephalosauridae

*Majungatholus*, new genus.

Type species: *Majungatholus atopus*, new species.

**Derivatio nominis:** The generic name is derived from the name of the region where the specimen was found and the Latin *tholus*, 'dome'.

**Diagnosis:** A single dome-like thickening of the frontal region; frontal dome thick, with highly irregular, rugose dorsal surface. Parietal without significant thickening, participating in a well developed parieto-squamosal shelf. Very large supratemporal fenestrae. Olfactory portion of the braincase long; olfactory lobes ventrally enclosed by bone.

*Majungatholus atopus*, new species.

**Holotype:** Skull roof consisting of frontals and incomplete parietals and anterior part of the braincase (Fig. 1). Muséum National d'Histoire Naturelle, Paris, MNHN.MAJ 4 (formerly in the collections of the Ecole des Mines, Paris).

**Locality and horizon:** 'Grès de Maevarano'<sup>9,12</sup>, Upper Cretaceous (probably Campanian), Majunga District, north-western Madagascar.

**Derivatio nominis:** The specific epithet is derived from the Greek *atopos*, 'strange' (or, in its original derivation, 'out of place'—a perhaps more appropriate meaning in the present context).

**Diagnosis:** As for the genus.

The specimen, a detailed anatomical description of which will be presented elsewhere<sup>9</sup>, evidently represents a dome-headed dinosaur referable to the Pachycephalosauridae. Like other pachycephalosaurids, it differs from the Ankylosauria in showing very long olfactory stalks and thickening of the cranial roof bones by upgrowth of the bones rather than by fusion of dermal ectopic elements<sup>14</sup>. Furthermore, in ankylosaurs the upper temporal fenestrae are always closed<sup>15</sup>.

*Majungatholus* represents a distinctive type of the dome-headed ornithischians; its single frontal dome and its very large upper temporal fenestrae are unique features. The earliest known pachycephalosaurid, *Yaverlandia bitholus*, from the Lower Cretaceous (Wealden) of the Isle of Wight<sup>1</sup>, shows some resemblance in having large supratemporal fenestrae and thickening restricted to the frontal region; it differs, however, in having a small dome on each frontal and in being much smaller. The only Upper Cretaceous pachycephalosaurid species showing a certain similarity is a flat-headed form referred to *Stegoceras* from the Campanian of Alberta, Canada<sup>1</sup>. In this species the supratemporal fenestrae are large and a parieto-squamosal shelf is well developed; the frontals, however, are but slightly thickened and the species is smaller than *Majungatholus atopus*. A peculiar feature only present in *Majungatholus* and the two Laurasian species mentioned above is a median circular depression on the postero-dorsal surface of the frontals.

*Majungatholus* can be derived from a *Yaverlandia*-like form and probably represents a lineage evolving in geographical separation from Northern Hemisphere pachycephalosaurids.

The presence of a pachycephalosaurid in Madagascar and of other 'Laurasian' dinosaur families in the Southern Hemisphere during the Late Cretaceous can be explained in two different ways. First, dispersal from Laurasia could be assumed as all these families have their greatest diversity and most primitive species in the Northern Hemisphere. The Tethyan sea belt separated the two super-continent<sup>16</sup> during the Cretaceous but it was at least in part a fairly shallow epicontinental sea and passage routes may have become available on several occasions. The exact locations of some of these connections may never be known as many potential areas have subsequently been affected by Cretaceous and Tertiary orogeneses. Cox<sup>4</sup> has suggested the existence of a filter route in the Central American or Caribbean region to account for the presence of a hadrosaur in Argentina. But it can also be argued that the existence of similar dinosaurs in both hemispheres is the result of vicariance events (the breakup of Pangea). The evidence presently available does not permit a choice between the two hypotheses. It is clear that faunal exchange by land connections between Laurasia and Gondwanaland was possible at least during the first half of the Cretaceous, possibly even at a later date.

The photographs for Fig. 1 were prepared by A. H. Coleman of Harvard University.

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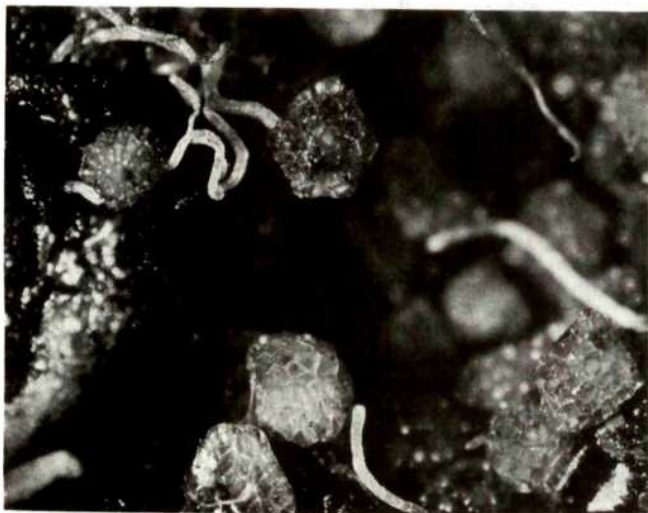
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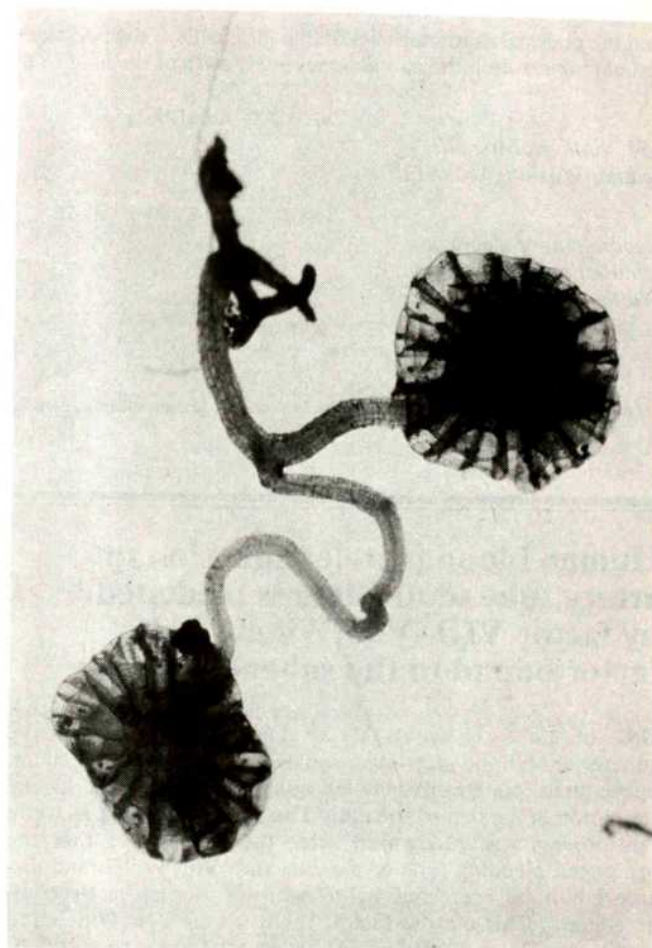
## Growth of host root establishes contact with parasitic angiosperm *Boschniakia hookeri*

MEMBERS of the family Orobanchaceae are root parasites attached underground to suitable hosts. In some cases attachment is effected by special mechanisms of the parasite. For example, the mature seed of *Orobanche*, stimulated chemotropically by diffusion of compounds from the host, germinates and its extending radicle establishes contact with the host<sup>1,2</sup>. We report here a previously unknown mechanism in *Boschniakia hookeri*, a perennial parasite found only on the roots of certain members of the Ericaceae in a narrow westerly distribution on the North American continent. We found that the connection of the parasite to the host is due ultimately to the behaviour of the host root, which grows through the testa of the parasite's seed into contact with the embryo.

We studied a population of *B. hookeri* growing in Kitsap County, Washington. Field observations showed that dispersal of the seeds depends in part on the properties of the seeds themselves, and in part on environmental factors. The mature seeds, as they are exposed in the ripe capsule, are relatively large ( $1.8 \pm 0.2$  mm), non-wettable, and of low specific gravity ( $0.2-0.25$ ). These properties result from the structure of the testa, a light, dry, honeycomb-like sheath surrounding the small embryo (about  $0.4 \times 0.3$  mm). Thus the seeds are easily displaced and chance movements (rain drops, animal contact) release them



**Fig. 1** Duff taken from below *Gaultheria shallon*. Many seeds of *Boschniakia hookeri* are present, as well as young roots of *Gaultheria*. The characteristic outer layer of deeply pitted cells of the testa (seed coat) makes it easy to recognise the *Boschniakia* seeds.



**Fig. 2** Branched roots of *Gaultheria shallon* with the root-tips growing through the outer testa cells towards the embryo. Material identical with seeds in Fig. 1.

from the capsule. Further dispersal is probably mainly through natural pores in the soil, or through tunnels made by small mammals (such as moles and mice). Occasional underground hoarding of seeds by mammals may also be a factor, although of secondary importance. Apparently the site of germination and the resulting tuber is determined merely by where seed and host root meet. Some tubers were found just beneath the surface, while in other cases tubers were as much as 25 cm below the surface—a depth of 10–15 cm was most common.

The embryo does not contain specialised organs<sup>3</sup> and apparently does not take an active part in the contact with the root. Connection is established by the young root-tip of the host, growing into and through the large outer cells of the testa (Figs 1, 2) until it presses against the embryo of the parasite. Later, the tissues of the parasitic embryo and the host merge. Cell division of the seed results in the formation of a tuber, in which vascular and meristematic tissues differentiate. This process can occur within 2–4 months from the time when the root and seed first come into contact. Apparently germination is not strictly dependent on the season, for young tubers are found in a continuous series of sizes, with a very close correlation between their dimensions and the diameter of the host roots.

The attraction between host root and parasite seems highly effective, although unexplained. For example, in germination experiments up to 75% of seeds placed on the roots of young *Arctostaphylos uva-ursi* were later found with the host roots growing into them. It is possible that the host roots are attracted by exudates from the embryo or testa. But as host root-tips can penetrate non-living organic particles (such as fragments of tree bark) and inorganic particles in the soil (such as artificially introduced Perlite, a heat-expanded volcanic glass), it may be



that the physical characteristics of the outer cells of the parasite's testa alone result in the contact-seeking growth of the host root.

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## Human blood platelet adhesion to artery subendothelium is mediated by factor VIII–Von Willebrand factor bound to the subendothelium

ONE of the early events in the haemostatic process is the adherence of blood platelets to collagen of the subendothelium. Subsequent platelet-to-platelet adhesion then leads to the formation of a haemostatic plug. The role of a plasma factor in this process was recognised after the discovery<sup>1,2</sup> that the prolonged bleeding time of patients with Von Willebrand disease could be corrected by transfusion of plasma or cryoprecipitate. This plasma factor, then named Von Willebrand factor, was later identified as factor VIII (anti-haemophilic

factor A (ref. 3)). The current concept is that Von Willebrand factor and factor VIII are present in a complex, which can be dissociated in certain conditions. Despite this progress, little is yet known about the role of the factor VIII–Von Willebrand factor complex (factor VIII–VWF) in the haemostatic plug formation. Tschopp *et al.* showed that the Von Willebrand factor was required for normal adhesion of platelets to rabbit subendothelium in a perfusion chamber using blood from patients with Von Willebrand disease<sup>4</sup>. The role of factor VIII–VWF in platelet adhesion is also important for the development of atherosclerosis. In this process, adherence of platelets is followed by degranulation and concomitant secretion of factors causing intimal migration and proliferation of smooth muscle cells<sup>5,6</sup>. Defective adherence of platelets was shown to protect against experimental atherosclerosis<sup>7,8</sup>. We present here evidence that factor VIII–VWF binds to subendothelium of human arteries and that subendothelium-bound factor VIII–VWF mediates the platelet adhesion.

A perfusion chamber as developed by Baumgartner<sup>9</sup> was used to study the effect of factor VIII–VWF on platelet adhesion to subendothelium, but using components of human origin only. Blood from healthy donors was anticoagulated with citrate. Platelets were washed in Krebs–Ringer solution, labelled<sup>10</sup> with <sup>51</sup>CrO<sub>4</sub> and incubated with aspirin to prevent platelet–platelet interaction<sup>11</sup> (for details see Fig. 1). Factor VIII–VWF was isolated from human cryoprecipitate<sup>12</sup> and labelled with <sup>125</sup>I by the lactoperoxidase method<sup>13</sup>. Subendothelium of human postmortem renal arteries after the first bifurcation was used; arteries with macroscopically observable atherosclerotic lesions were discarded. Perfusions were carried out at a shear rate comparable to that in arteries<sup>14</sup>.

In the first set of experiments the specificity of the effect of factor VIII–VWF on the adhesion of <sup>51</sup>Cr-platelets was studied. After perfusion for 5 min at 37 °C with normal plasma containing red cells (haematocrit 40%) and <sup>51</sup>Cr-platelets ( $2.5 \times 10^5$  per



**Fig. 1** Light microscopical picture ( $\times 1,100$ ) of human subendothelium after perfusion with aspirin-treated platelets. No platelet interaction could be detected, only a monolayer of platelets covering the subendothelium. All arteries used had subendothelial fibrosis. Fixation, embedding, cutting and staining was after Baumgartner<sup>15</sup>. The arteries were obtained from autopsies usually 12 h after death. They were exposed to air for a few minutes to remove endothelial cells completely, and the lumen was wiped with a bud probe. Finally, the lumen was rinsed with 0.2 M Tris buffer (pH 7.4). On light microscopical examination no endothelial cells could be detected. The reconstituted blood used for the perfusions was anticoagulated with 19 mmol citrate (plasma concentration). Platelet-rich plasma (PRP) was obtained from whole blood by centrifugation (10 min at 190g, 20 °C). To one volume of PRP one volume of a Krebs–Ringer solution (4 mM KCl, 107 mM NaCl, 20 mM NaHCO<sub>3</sub> and 2 mM Na<sub>2</sub>SO<sub>4</sub>) containing 19 mM citrate and 0.5% glucose (pH 5.0) was added which gave a final pH of 6.1. Platelets were spun down (10 min, 500g, 20 °C) and resuspended in 2 ml Krebs–Ringer solution (with citrate and glucose, pH 6.1) four times. The second time 1  $\mu$ Ci CrO<sub>4</sub> per ml was added to the resuspended platelets (about  $10^6$  per  $\mu$ l) and centrifugation was carried out after 20 min at 20 °C. The third time, 10  $\mu$ M aspirin was added for 15 min at 37 °C. After the fourth washing over 97% of the <sup>51</sup>CrO<sub>4</sub> was intracellular. Platelet poor plasma (PPP) was obtained from PRP by centrifugation (15 min, 3,000g, 4 °C). Plasma contamination of the red cells was avoided by three washings in saline containing 2% glucose. The labelled platelets, PPP and the red cells were reconstituted to give  $2.5 \times 10^5$  platelets per  $\mu$ l plasma and a red cell concentration of approximately 40%. The pH was adjusted to 7.3. The perfusates were incubated for 5 min at 37 °C before the perfusions were carried out. A peristaltic tube pump (Verder) was used for the perfusions. The average flow rate was 135 ml min<sup>-1</sup> and the average shear rate was calculated to 805 s<sup>-1</sup>. All perfusions were carried out for 5 min at 37 °C. Immediately after perfusion the system was rinsed with 40 ml 0.2 M Tris buffer (pH 7.4). A segment of approximately 2.5 mm was cut from both ends of the artery. It was calculated that a 2.5-mm broad area of turbulence would occur where the flow met the edge of the vessel wall<sup>16</sup>. The remaining part ( $\sim 0.5$  cm<sup>2</sup>) was used for registration of <sup>51</sup>Cr (Trigamma 600, Baird Atomic).

**Table 1** Platelet adherence to subendothelium in media with and without factor VIII-VWF

	Platelet no. ( $\times 10^5$ ) per $\text{cm}^2$ subendothelium (mean $\pm$ s.d.)	
	Without addition of factor VIII-VWF	Addition of 1 U factor VIII-VWF per ml
Normal plasma	—	71.5 $\pm$ 11.0 (7)
Haemophilia A plasma	—	67.2 $\pm$ 5.8 (5)
Von Willebrand plasma*	28.8 $\pm$ 8.2 (11)	73.3 $\pm$ 8.7 (6)
Plasma after cryoprecipitation†	24.5 $\pm$ 7.6 (6)	70.2 $\pm$ 5.3 (5)
Human albumin solution (4.0%)	26.2 $\pm$ 8.7 (7)	77.5 $\pm$ 8.0 (5)
Dextran 40.000 solution (3.3%)	31.2 $\pm$ 3.1 (5)	60.3 $\pm$ 7.5 (5)

Number of perfusions in parentheses.

\* Factor VIII coagulant activity 14%, ristocetin cofactor assay 5% and factor VIII-related antigen 7%.

† Factor VIII coagulant activity 15%, ristocetin cofactor assay 1% and factor VIII-related antigen 15%.

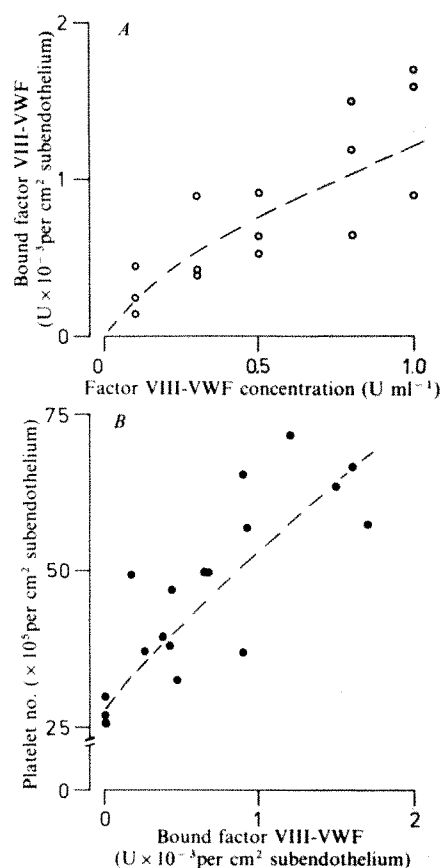
$\mu\text{l}$  plasma),  $70 \times 10^5$  platelets per  $\text{cm}^2$  subendothelium were detected. When normal plasma was substituted by plasma from patients with Von Willebrand disease or by supernatant plasma after cryoprecipitation, the platelet adhesion was markedly decreased. A similar decrease was found on substitution of normal plasma by solutions of human albumin or dextran, which indicated that no proteins other than factor VIII-VWF play a part in the platelet adhesion. Indeed, addition of factor VIII-VWF to these plasma substitutes corrected the platelet adhesion to the same level as obtained with normal plasma (Table 1). This normalisation was reached in a relatively small range (0.5–0.8 U per ml of factor VIII-VWF as measured in the ristocetin cofactor assay<sup>17</sup>).

The mechanism by which factor VIII-VWF promotes the adhesion of platelets to the subendothelium was studied in the following way. First, perfusions (5 min, 37 °C) were carried out using solutions of human albumin and factor VIII-VWF (1 U  $\text{ml}^{-1}$  including 10%  $^{125}\text{I}$ -factor VIII-VWF). These experiments indicated a binding of  $10^{-3}$  U factor VIII-VWF per  $\text{cm}^2$  subendothelium. Then, double-perfusion experiments were carried out to study the influence of bound factor VIII-VWF on the platelet adhesion. Subendothelium was perfused with human albumin solutions containing various amounts of  $^{125}\text{I}$ -factor VIII-VWF, followed by a second perfusion with red cells and  $^{51}\text{Cr}$ -platelets in albumin solutions without factor VIII-VWF. Figure 2 shows the relationship between the concentration of factor VIII-VWF and the binding of this protein to the subendothelium (A) and the dependence of the platelet adhesion on the amount of factor VIII-VWF bound (B). A nearly normal adhesion was obtained after binding of about  $10^{-3}$  U factor VIII-VWF per  $\text{cm}^2$  subendothelium ( $4 \times 10^9$  molecules per  $\text{cm}^2$ ). Removal of factor VIII-VWF from the subendothelium during the second perfusion could not be detected. It should be mentioned that even complete desorption would yield a negligible concentration of factor VIII-VWF in the second perfusate.

From these experiments, carried out with human components in conditions which mimic the *in vivo* situation after vessel wall injury, we conclude (1) that for normal adhesion factor VIII-VWF is the only plasma protein necessary, and (2) that factor VIII-VWF bound to subendothelium is a highly effective inducer of platelet adhesion.

The results strongly suggest that binding of factor VIII-VWF to subendothelium is an important step in the first phase of the haemostatic plug formation. This is supported by ultrastructural studies of haemostatic plugs in Von Willebrand patients<sup>18,19</sup> showing a disturbed adherence of platelets to the vessel lips and the presence of free platelet aggregates in the wounds. The effect of factor VIII-VWF on platelet adhesion may also be important for the development of atherosclerosis, as was concluded by Fuster *et al.*<sup>8</sup>, who showed that pigs with severe Von Willebrand disease are much less susceptible to atherosclerosis than control pigs.

Furthermore, the present results are interesting in view of the synthesis of factor VIII-VWF-related antigen (with Von



**Fig. 2** Factor VIII-VWF accumulation and the subsequent platelet adhesion on human subendothelium in a double-perfusion experiment. A, Factor VIII-VWF bound to the subendothelium in the first perfusion (○) with different factor VIII-VWF concentrations. B, Number of platelets adhered to the subendothelium in the second perfusion (●), with the corresponding amount of pre-bound factor VIII-VWF. Dashed curves are power curve fits according to the formula  $y = ax^b + c$ , where in A:  $a = 1.23$ ,  $b = 0.70$ ,  $c = 0.0$  and the correlation coefficient  $r = 0.85$ , and in B:  $a = 26.0$ ,  $b = 0.86$ ,  $c = 26.7$  and  $r = 0.84$ . In the first perfusion a Krebs-Ringer solution containing 19 mM citrate, 2.4 mM  $\text{CaCl}_2$ , 0.5% glucose and 4% human albumin (recrystallised, Sigma A 9511) with different concentrations of purified factor VIII-VWF was perfused for 5 min, at 37 °C. 10% of the purified factor VIII-VWF was labelled with  $^{125}\text{I}$ . After the first perfusion, the system was rinsed with 135 ml 0.2 M Tris buffer (pH 7.4). The second perfusion (5 min, 37 °C) was then started immediately. This perfusate contained the Krebs-Ringer solution as used in the first perfusion but without factor VIII-VWF. Platelet and red cell concentrations were similar to the concentrations, as indicated in Fig. 1 legend. After the second perfusion, the system was rinsed with 40 ml 0.2 M Tris buffer (pH 7.4). The middle part of the artery was cut from the rod and used for registration of  $^{51}\text{Cr}$  and  $^{125}\text{I}$ .



Willebrand activity but without coagulant activity) in the endothelial cells<sup>20</sup>. On vessel wall injury, this antigen may be bound to the subendothelium and subsequently mediate the adhesion of platelets. A local haemostatic effect of factor VIII-VWF-related antigen present in the vessel wall has been suggested by Bloom *et al.*<sup>21</sup> on the basis of immunohistochemical studies of the intima and by Holmberg *et al.*<sup>22</sup> who reported the absence of this antigen in the vessel wall of severely affected Von Willebrand patients.

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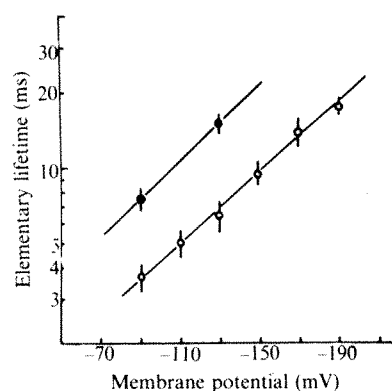
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## Calcium conductance of acetylcholine-induced endplate channels

SEVERAL lines of evidence indicate that an influx of  $\text{Ca}^{2+}$  ions accompanies transmitter activation of the postsynaptic membrane at the neuromuscular junction<sup>1–5</sup>. We report here that we have now investigated the elementary characteristics of the  $\text{Ca}^{2+}$  current by bathing muscles in  $\text{CaCl}_2$  solutions containing no  $\text{Na}^+$  ions, so that  $\text{Ca}^{2+}$  is the only ion available to carry any appreciable inward current<sup>1,3,6,7</sup>. The single channel current ( $i$ ) and mean lifetime ( $\tau$ ) of the postsynaptic channels were then determined by the technique of noise analysis<sup>8,9</sup>. It was found that  $\tau$  in the  $\text{Ca}^{2+}$  solution was shorter than in normal Ringer, but that the voltage dependence of  $\tau$  was unchanged. The elementary current showed a non-linear voltage dependence, unlike the linear dependence in normal solution<sup>8</sup>.

Experiments were carried out on the frog (*Rana temporaria*) sartorius muscle, at a temperature of 5–6 °C. The muscle was strongly stretched to reduce contraction and its associated artefacts. The normal bathing solution contained (in mM): NaCl, 120; KCl, 2;  $\text{CaCl}_2$ , 1.8; HEPES, 4 (pH 7.2). Calcium solutions contained:  $\text{CaCl}_2$ , 82 or 160; KCl, 2; HEPES, 4 (pH 7.2): care was taken to avoid contamination with  $\text{Na}^+$ . A standard two-point voltage clamp was used at the endplates, and a third external micropipette filled with 2 M acetylcholine (ACh) was used to apply ACh ionophoretically to the endplate. A fast



**Fig. 1** Membrane potential dependence of the ACh-induced single channel lifetime,  $\tau$ , measured from muscles bathed in normal Ringer solution (●) and in isotonic or hypertonic  $\text{Ca}^{2+}$  solutions (○). No differences were seen in  $\tau$  between isotonic and hypertonic  $\text{Ca}^{2+}$  solutions, and the results shown are pooled data from both. Lines were fitted by eye, and error bars give  $\pm 1$  s.d. Five endplates were examined in Ringer solution, and seven in  $\text{CaCl}_2$  solutions. Values were obtained during steady ionophoretic application of ACh to voltage-clamped endplates, by fitting the membrane current fluctuation spectra to a lorentzian curve. Background spectra were subtracted before fitting.  $\tau$  was calculated from the half power frequency  $f_c$  of the spectra as  $\tau = 1/(2\pi f_c)$ . About 15 noise segments of 512 points were obtained at each potential, at a digitisation rate of 500 or 1,000 Hz, and were used to compute an average spectrum.

Fourier transform method was used to analyse the frequency components of the current fluctuations during steady ACh application, and  $i$  and  $\tau$  were determined as previously described<sup>8,9</sup>.

Records were usually obtained from several fibres in normal Ringer solution, and then from the same, and additional fibres, after changing to  $\text{Ca}^{2+}$  solution. Resting membrane potentials were higher in isotonic and hypertonic  $\text{Ca}^{2+}$  solutions (mean values: normal Ringer  $85.1 \pm 5.8$  mV,  $\text{Ca}^{2+}$  solution  $103 \pm 7.2$  mV: all deviations are  $\pm 1$  s.d. unless otherwise stated), and the input resistance of the fibres was increased (normal Ringer  $\sim 350$  k $\Omega$ ;  $\text{Ca}^{2+}$  solution  $1.32 \pm 0.38$  M $\Omega$ ). Pipettes with large tip diameters were used to reduce background noise, and the values given above may be artificially low because of fibre damage during penetration. The frequency of miniature endplate potentials was elevated after changing to  $\text{Ca}^{2+}$  solution, but fell to nearly zero after 2–3 h, when ACh noise recordings could be made. Miniature endplate currents (m.e.p.cs) were reduced in duration and size in  $\text{Ca}^{2+}$  solution (see also ref. 3), mean amplitudes at  $-130$  mV being  $2.86 \pm 0.39$  nA in normal Ringer, and  $0.97 \pm 0.37$  nA in  $\text{Ca}^{2+}$  solution.

In normal Ringer, ionophoretic application of large doses of ACh to the endplate caused local contractions, and these became much larger in isotonic  $\text{Ca}^{2+}$  solution. For example, a barely visible contraction, involving two sarcomeres, was seen with a synaptic charge flux of about 1 nC. Because of this difficulty in avoiding movement artefacts, some experiments were carried out in hypertonic  $\text{Ca}^{2+}$  solution (160 mM  $\text{CaCl}_2$ ), which helped to reduce contraction. To guard against the possibility that part of the synaptic current was carried by an influx of  $\text{Cl}^-$  ions<sup>7</sup>, recordings were made from two fibres in a bathing solution of 82 mM Ca-cyclamate. M.e.p.cs were recorded in this solution, with the same amplitudes as in 82 mM  $\text{CaCl}_2$  solution, and there was little difference in reversal potential. Also  $\text{K}^+$  ion influx made no appreciable contribution to the synaptic current, as no significant changes in equilibrium potential or single channel currents were seen in fibres bathed in a solution containing only 160 mM  $\text{CaCl}_2$ .

A variety of divalent cations in addition to  $\text{Ca}^{2+}$  are able to carry a synaptic current. We have recorded m.e.p.cs and ACh-induced currents from fibres bathed in isotonic solutions of  $\text{MgCl}_2$ ,  $\text{MnCl}_2$ ,  $\text{SrCl}_2$  and  $\text{CoCl}_2$  (all solutions also contained 2 mM KCl and 4 mM HEPES). This contrasts with the

behaviour in several other systems, where  $Mg^{2+}$ ,  $Mn^{2+}$  and  $Co^{2+}$  are known to block  $Ca^{2+}$  fluxes<sup>10-13</sup>. The drug D600 has also been shown to block  $Ca^{2+}$  fluxes in a variety of preparations<sup>11-13</sup>. We found that at a concentration of  $200 \mu g ml^{-1}$  D600 decreased the frequency of m.e.p.s in isotonic  $Ca^{2+}$ , and did not abolish the ACh sensitivity of the muscle membrane. However, with ionophoretic ACh application only brief transient responses could be recorded, and a period of a few minutes was required for recovery of the response to a second pulse. This effect of D600, which was also present in normal Ringer, may be attributable to a blocking of open channels, or to an increase in desensitisation.

Figure 1 shows the voltage dependence of mean lifetime ( $\tau$ ) of ACh-induced channels in  $Ca^{2+}$  solution, plotted on semi-logarithmic coordinates. The data are fitted well by a straight line, indicating that  $\tau$  increases exponentially with membrane hyperpolarisation. The voltage constant (potential shift to give an  $e$ -fold change in  $\tau$ ) was 62 mV.  $\tau$  increases exponentially with hyperpolarisation in normal Ringer<sup>8</sup>, and the  $Ca^{2+}$  data closely parallel a line drawn through control measurements in normal Ringer, although shifted in a hyperpolarised direction by about 46 mV. The shortening of  $\tau$  in  $Ca^{2+}$  solution is surprising, as an increase in  $Ca^{2+}$  concentration from 1 to 10 mM has been reported to slow the decay phase of m.e.p.s<sup>14</sup>. However, we have been unable to confirm these results, and found that this increase in  $Ca^{2+}$  concentration produced in some experiments a shortening of m.e.p.c. decay, and in others no detectable change.

Figure 2 shows the voltage dependence of the elementary current, derived from noise analysis, in hypertonic  $Ca^{2+}$  solution. The relationship is not linear throughout, indicating that the elementary conductance ( $\gamma$ ) varies with membrane potential. Closely similar behaviour was seen in experiments using isotonic  $Ca^{2+}$  solution. This is unlike the situation in normal Ringer, where  $\gamma$  is independent of potential<sup>8</sup>, and in our experiments had a value of  $29.3 \pm 2.7$  pS (s.e.m.). In hypertonic  $Ca^{2+}$  solution the voltage dependence of  $i$  was approximately linear at potentials more hyperpolarised than -110 mV, and the slope of this segment gave a value for  $\gamma$  of 6.25 pS. Noise measurements at potentials between equilibrium and -100 mV were very difficult to obtain, but an average value for  $\gamma$  of  $\sim 2.5$  pS was estimated for this segment.

Our results confirm previous observations<sup>1-5</sup> showing that activation of the postsynaptic membrane with ACh causes an increase in permeability to  $Ca^{2+}$  ions, and further indicate that  $Mg^{2+}$ ,  $Mn^{2+}$ ,  $Sr^{2+}$  and  $Co^{2+}$  ions are also able to cross the channels induced by ACh. However, the conductances for these ions are small, and at -90 mV, for example, the elementary conductances in both isotonic  $MgCl_2$  and  $CaCl_2$  solutions are about 10 times less than in normal Ringer.

Divalent ions might cross the endplate membrane (1) nonspecifically through the 'ordinary' channels normally used

by  $Na^+$  and  $K^+$ , or (2) through a separate  $Ca^{2+}$ -specific channel. The apparently identical voltage dependence of  $i$  for  $Ca^{2+}$  and  $Na^+$  currents, together with the failure of  $Mg^{2+}$ ,  $Mn^{2+}$ ,  $Co^{2+}$  and D600 to block the m.e.p.s, favour (1), but do not entirely exclude (2). The reason for the differences in conductance properties in Na- or Ca-Ringer is not clear, and the non-linear dependence of the elementary  $Ca^{2+}$  current on membrane potential is not predicted by the Goldman-Hodgkin-Katz model of ion permeation<sup>15-18</sup>. However, differences also exist between various divalent ions themselves, and we find that, unlike  $Ca^{2+}$ , the elementary  $Mg^{2+}$  current has a linear voltage dependence over the range examined (-50 to -130 mV).

The decrease in channel lifetime in  $Ca^{2+}$  solutions is in the opposite direction to that expected from the effect of  $Ca^{2+}$  ions in screening negative surface charges on the muscle membrane<sup>14</sup>, and suggests that  $Ca^{2+}$  ions have an additional effect on the channel. It may be that in addition to direct effects of various ions on the receptors and their environment, the lifetime of transmitter-induced channels is influenced by the nature of the ions which permeate them<sup>19</sup>.

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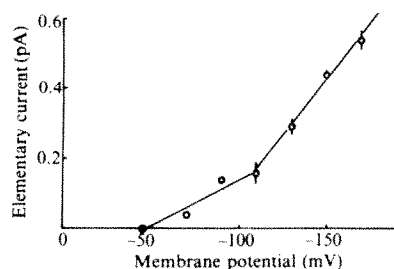
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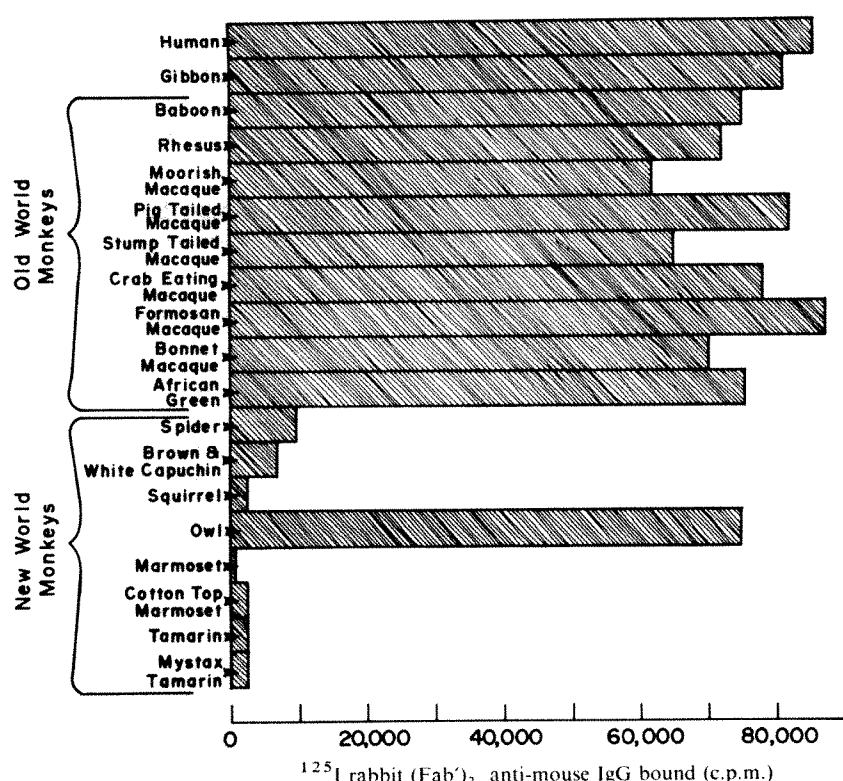
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**Fig. 2** Membrane potential dependence of the single channel current  $i$ , measured from fibres bathed in 160 mM  $CaCl_2$  solution. Lines were fitted by eye. Points shown with error bars are means from five fibres, and bars are  $\pm 1$  s.e.m. Points at -90 and -70 mV are measurements from single fibres. The equilibrium potential (●) was measured from the reversal potential of the ACh response in seven fibres, and was -44.4 mV (s.d.  $\pm 1.1$  mV). Elementary currents were calculated from the total spectrum variance, after subtraction of background variance. The alternative technique of computing  $i$  from the zero frequency asymptote of the spectra gave values in good agreement.

## Anti-HLA-A,B,C monoclonal antibodies with no alloantigenic specificity in humans define polymorphisms in other primate species

THE study of evolutionary relationships by immunological comparison of homologous proteins from different species has been limited by the complexity and variability of conventional heteroantisera<sup>1</sup>. These problems can be avoided by using selected panels of monoclonal antibodies, specific for single antigenic determinants, which would enable detailed immunochemical descriptions of homologous proteins to be made<sup>2</sup>. The human HLA-A,B,C antigens and their homologues provide a particularly interesting system for this type of analysis<sup>3</sup>. Although they are extraordinarily polymorphic within species, many features of their structure, including association with  $\beta_2$ -microglobulin, have been highly conserved during evolution<sup>4-6</sup>. A preliminary survey showed that monoclonal antibodies with specificity for the HLA-A,B,C- $\beta_2$ -microglobulin complex reacted with primate species but not with non-primates<sup>7</sup>. We have therefore



**Fig. 1** Indirect trace binding of W6/32 antibody to lymphocytes of different primate species. The assay was basically as described in ref. 14. Peripheral blood lymphocytes ( $5 \times 10^5$ ) in a volume of 50  $\mu$ l were incubated with 50  $\mu$ l of a 1/100 dilution of ascites fluid from mice carrying the W6/32 hybrid cell as a tumour, for 1 h at 4°C. After three washes the cells were incubated with  $2 \times 10^6$  c.p.m. of  $^{125}$ I-labelled rabbit (Fab')<sub>2</sub> anti-mouse IgG for 1 h at 4°C, washed four times and counted for radioactivity.

concentrated on comparing primate species with a panel of anti-A,B,C monoclonal antibodies. We report here the finding that two different anti-HLA-A,B,C monoclonal antibodies with no demonstrable polymorphic specificity in humans define polymorphisms in owl and spider monkeys, thus demonstrating the potential of these reagents for fine structure antigenic mapping.

Results obtained from a limited number of species indicated that the anti-HLA-A,B,C monoclonal antibody W6/32 reacted with apes and Old World monkeys whereas the anti- $\beta_2$ -microglobulin monoclonal antibody BBM1 only reacted with the higher apes, chimpanzee and gorilla<sup>7,8</sup>. The indirect binding of W6/32 to peripheral lymphocytes from individual animals of 19 primate species is shown in Fig. 1. Positive reactions were found with all species of Old World monkeys and the only ape (gibbon) tested. All New World species, with the exception of the owl monkey, were negative. The BBM1 antibody, as expected (ref. 7, and data not shown), was negative against all non-human species tested.

To confirm the presence of the W6/32 antigenic determinant on owl monkey lymphocytes four animals were individually tested. Three gave negative reactions and the single animal tested in the original screen was still positive. Subsequent typing of 40 owl monkeys showed 11 positive and 29 negative. When lymphocytes from W6/32 positive and negative animals were assessed for their capacity to bind W6/32 antibody (Fig. 2), positive animals gave titration curves indistinguishable from human and rhesus lymphocytes, in contrast to negative animals, which gave no significant binding at any cell number and were indistinguishable from spider monkey lymphocytes used as the negative control.

The owl monkey is widely distributed throughout South America, and regional variations in coat colour and chromosomal morphology have been described<sup>9</sup>. The polymorphic reaction pattern of W6/32 antibody was found to correlate exactly with chromosomal differences. Nine animals with karyotype VI from Bolivia and two with karyotype VII from Peru were all positive, six animals with karyotype I from Brazil and 23 animals with karyotypes II, III, IV and V from Columbia were all negative (Fig. 3 and Table 1).

Other anti-HLA-A,B,C monoclonal antibodies which showed no polymorphic reactions in humans<sup>10</sup> were screened

against owl monkey lymphocytes of different karyotype. Three different reaction patterns were seen: (1) negative on all cells, (2) identical to W6/32 antibody, and (3) a polymorphic reaction pattern different from the W6/32 antibody. Three antibodies of the last class were identified which all gave similar reaction patterns. Positive reactions were seen with all animals of karyotypes I, II, III, IV, V and VII whereas polymorphic reactions were found with animals of karyotype VI (Table 1). One antibody (PA2.5) was selected for further study and its specificity on all human cells confirmed. PA2.5 antibody reacted equally well with a panel of 20 HLA-A, B, C-bearing human B-cell lines and this binding was equally inhibitable by purified preparations of both HLA-A locus (A2 and A28) and HLA-B locus (B7 and B40) antigens<sup>11</sup>.

As the initial screen (Fig. 1) with the W6/32 antibody against different species involved single animals and by chance, an owl monkey of karyotype VII had been selected, it became important to re-evaluate the reactions on other species. Groups of three New World species (squirrel, spider, cebus) and a single Old World species (rhesus) were tested against W6/32 and PA2.5 antibodies and the results are shown in Table 1. The original conclusions concerning the W6/32 antigenic determinant were confirmed. It was absent on all cebus, squirrel and spider monkeys and present on all rhesus monkeys tested. The

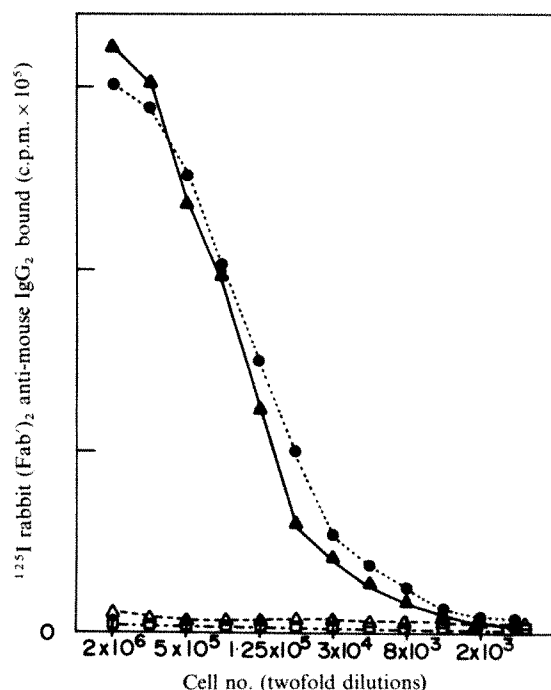
**Table 1** Indirect binding reactions of W6/32 and PA2.5 antibodies on primate lymphocytes

Species	No. of animals tested	W6/32		PA2.5	
		Positive	Negative	Positive	Negative
Spider monkey	12	0	12	8	4
Owl monkey (karyotype VI)	9	9	0	3	6
Owl monkey (karyotype I, II, III, IV, V)	29	0	29	29	0
Owl monkey (karyotype VII)	2	2	0	2	0
Squirrel monkey	20	0	20	20	0
Cebus (brown and white capuchin) monkey	15	0	15	15	0
Rhesus monkey	14	14	0	14	0



PA2.5 antibody gave positive reactions with all rhesus, squirrel and cebus monkeys but was polymorphic in the spider monkey.

The polymorphisms defined by the two monoclonal antibodies in the owl monkey were quite distinct. W6/32 antibody reactions correlated exactly with known differences in karyotype, coat colour and geographical origin. This result is similar to that found for wild mice where individual demes of geographically separated populations had characteristic H-2 antigens<sup>12</sup>. The PA2.5 antibody defined a polymorphism within a presumably interbreeding group of animals that were identical for karyotype, coat colour and W6/32 antibody reactivities. Similarly, the polymorphism defined by the PA2.5 antibody in the spider monkey was not correlated with other known parameters.

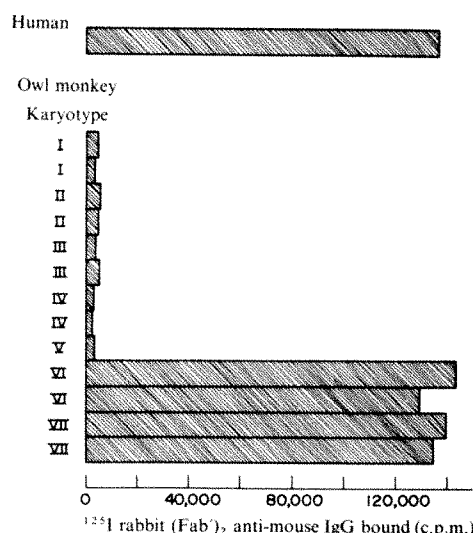


**Fig. 2** Indirect trace binding of W6/32 antibody to human, spider monkey and owl monkey lymphocytes as a function of cell number. Assays were as described in the Fig. 1 legend except different cell numbers were used. ●, Owl monkey with karyotype VI; □, owl monkey with karyotype I; ▲, human used as a positive control; △, spider monkey used as negative control.

It is not unexpected that non-polymorphic anti-HLA-A,B,C monoclonal antibodies define polymorphism in other primate species. Highly conserved regions of HLA-A,B,C glycoproteins will not be immunogenic in mice, and it is likely that monoclonal antibodies made in mice will recognise parts of the molecule that can tolerate amino acid substitutions without loss of function and thus have the potential for polymorphism within a species.

Most non-polymorphic anti-HLA-A,B,C monoclonal antibodies we have produced bind to cells of non-human primate species. Comparison of their reactions with different species has provided a simple way of defining different antigenic determinants of the HLA-A,B,C- $\beta_2$ -microglobulin complex. The W6/32 antibody defines a determinant generally restricted to apes and Old World monkeys, the PA2.5 antibody defines a determinant present on apes, Old World monkeys and most New World monkeys. These results indicate that both antigenic determinants originated before the divergence of New and Old World monkeys and it will be interesting to see if they can be detected in tree shrews and the more primitive species of primates.

The high frequency with which antibodies of similar specificity are produced suggests that the number of antigenic determinants is small. This is probably a reflection of the  $\approx 70\%$



**Fig. 3** Indirect trace binding of W6/32 antibody to lymphocytes from owl monkey with seven different karyotypes. Assays were as described in the Fig. 1 legend.

amino acid sequence homology between HLA-A,B,C glycoproteins and the H-2D,K glycoproteins of the mice used to make the antibodies<sup>5,6</sup>. The finding that HLA-A,B,C antigens and their homologues in higher primates are antigenically similar in comparison to mouse indicates a very high degree of amino acid sequence homology. The equivalence with which many anti-HLA monoclonal antibodies bind to cells of non-human primate species and the use of insolubilised W6/32 antibody in the purification of HLA-A,B,C antigens<sup>13</sup> suggest that the homologous antigens of other species may be purified by this method and made available for structural characterisation.

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## Functional separation *in vivo* of both antigens encoded by H-2 subregion and non-H-2 loci

ANTIGENS coded for by the H-2K, H-2D and H-2I region of the major histocompatibility complex (MHC) of the mouse initiate different *in vitro* responses. I region-coded antigens activate mainly Lyt 1<sup>+</sup> T cells to produce a proliferative response in mixed lymphocyte cultures (MLC), whereas K and D region-coded antigens predominantly stimulate Lyt 2<sup>+</sup> T cells to become cytolytic effector cells<sup>1</sup>. Proliferative responses in MLC and generation of cytolytic T cells *in vitro* can also be induced by minor histocompatibility antigens. For example, Mls-locus products can induce positive MLC responses, but not the generation of cytolytic T cells<sup>2</sup>, whereas H-Y antigen does give rise to anti-H-Y cytotoxic T-cell responses *in vitro*, following *in vivo* priming<sup>3</sup>. Functional *in vivo* studies have shown that H-2K, H-2D and H-2I differences can account for graft rejection<sup>4</sup> and for mortality in graft-versus-host (GvH) reactions<sup>5</sup>. Differences in only minor histocompatibility antigens are sufficient to cause graft rejection<sup>6</sup>, and lethal GvH reaction after allogeneic bone marrow transplantation<sup>7</sup>. Similarly, immunisation with only H-2 or only non-H-2 antigens can induce a state of delayed-type hypersensitivity (DTH) to the immunising antigen, which can be measured with the footpad swelling test<sup>8</sup>. So far, such *in vivo* experiments have shown little or no discrimination between

responses to H-2 subregion antigens and responses to non-H-2 antigens. We have used a DTH assay to study the occurrence of T-effector cells after *in vivo* immunisation with different histocompatibility antigens. We show here that DTH T-effector cells generated in GvH and host-versus-graft (HvG) reactions are specific for largely different sets of histocompatibility antigens, with selective stimulation by H-2I and Mls-locus antigens in GvH conditions.

We have developed a simple DTH assay which can be used to measure the development of T-effector cells induced by histocompatibility antigens during a GvH reaction<sup>9,10</sup>. The assay is based on secondary transfer of lymphoid cells from animals undergoing a GvH reaction, and subsequent testing of the secondary recipients for DTH reactivity to the histocompatibility antigens which evoked the GvH reaction. Briefly, lymphoid cells from mice which have been irradiated and reconstituted with allogeneic spleen cells, are transferred intravenously (i.v.), at various intervals after reconstitution, into normal secondary recipients syngeneic to the original spleen cell donor. The secondary recipients are challenged in the right hind foot with  $2 \times 10^7$  spleen cells syngeneic to the original irradiated recipients. The DTH response to this challenge is measured as the difference in thickness of the hind feet 24 h later. The specific increase in foot thickness is calculated as the relative increase in foot thickness of the immune mice minus the relative increase in foot thickness of control mice which have only received the challenge. The swelling of challenged control mice varies between 12 and 20%. Using this assay we studied the capacity of

**Table 1** Anti-host immune reactivity in lethally irradiated recipients after spleen cell transplantation across H-2-subregion differences

Inoculum	Recipient	H-2 subregion coding for immunising antigen*	Challenge	H-2 subregion coded antigens of cells used for challenge	DTH Response
B10.A	B10.AQR	K	B10.AQR	K	3.2 ± 1.6
B10.AQR	B10.A × B10.T(6R)	K + I	B10.A × B10.T(6R)	K + I	35.5 ± 1.7
B10.AQR	B10.A × B10.T(6R)	K + I	B10.T(6R)	I	32.2 ± 2.9
B10.AQR	B10.A × B10.T(6R)	K + I	B10.A	K	3.4 ± 3.0
A.TH	A.TL	I(+Tla)	A.TL	I(+Tla)	31.4 ± 2.6
A.TH	A.SW	D(+Tla)	A.SW	D(+Tla)	-0.3 ± 1.4

GvH reactions were elicited by i.v. injection of  $5 \times 10^7$  spleen cells (inoculum) into lethally irradiated recipient mice. Recipients were lethally irradiated (750 rad) within 4 h before reconstitution with the spleen cell inoculum; 5 d after reconstitution two-thirds of the total cell yield obtained from spleen, inguinal, axillary and mesenteric lymph nodes of a recipient mouse were transferred i.v. to a secondary recipient which was syngeneic to the original spleen cell donor. Lymphoid cells from all primary recipients of a particular combination were pooled before secondary transfer. Challenge was carried out with  $2 \times 10^7$  (50 µl) spleen cells (treated with mitomycin C (Kyowa Hakko Kogyo)  $100 \mu\text{g ml}^{-1}$  for 30 min at 37 °C) injected s.c. into the instep of the right hind foot of the secondary recipients. At 24 h after challenge DTH responses were measured. Control mice only received a challenge dose. The swelling of these control mice was 12–20%. DTH responses are expressed as the specific percentage increase in foot thickness, as described previously<sup>9</sup>. Figures represent the arithmetic mean ± 1 s.e.m. of five mice.

\* H-2-subregion difference between spleen cell donor and irradiated recipient mice. The origin of H-2 subregions (K, I-A, I-B, I-C, D) for the strains are as follows: A.TH, ssssd; A.SW, sssss; A.TL, skkdd; B10.A, kkkdd; B10.AQR, qkkdd; B10.T(6R), qqqdd.

**Table 2** Anti-host immune reactivity in lethally irradiated recipients after spleen cell transplantation across Mls-locus differences

Inoculum	Recipient	Peak MLC response	Challenge	Mls locus-coded antigen of cells used for challenge	Peak DTH response
BALB/c	BALB/c × DBA/2	24,000 ± 850 (4)	DBA/2	Mls <sup>a</sup>	25.5 ± 0.7 (5)
BALB/c	BALB/c × DBA/2	—	B10.D2	Mls <sup>b</sup>	0.5 ± 1.2 (5)
DBA/2	BALB/c × DBA/2	630 ± 280 (3)	BALB/c	Mls <sup>b</sup>	10.6 ± 2.2 (3)
AKR/FuRdA	C3H/f	22,300 ± 1300 (3)	C3H/f	Mls <sup>c</sup>	25.8 ± 1.8 (7)
AKR/FuRdA	C3H/f	—	B10.BR	Mls <sup>b</sup>	2.9 ± 1.1 (7)
C3H/f	AKR/FuRdA	38,000 ± 2900 (4)	AKR/FuRdA	Mls <sup>a</sup>	26.7 ± 2.0 (7)
C3H/f	AKR/FuRdA	—	B10.BR	Mls <sup>b</sup>	1.9 ± 1.7 (7)

GvH reactions were elicited by i.v. injection of  $5 \times 10^7$  spleen cells (inoculum) into lethally irradiated recipient mice. The designation for H-2 haplotype and Mls locus for the strains are as follows: BALB/c, H-2<sup>d</sup>, Mls<sup>b</sup>; DBA/2, H-2<sup>d</sup>, Mls<sup>a</sup>; BALB/c × DBA/2, H-2<sup>d/d</sup>, Mls<sup>b/a</sup>; B10.D2, H-2<sup>d</sup>, Mls<sup>b</sup>; AKR/FuRdA, H-2<sup>k</sup>, Mls<sup>a</sup>; C3H/f, H-2<sup>k</sup>, Mls<sup>c</sup>; B10.BR, H-2<sup>k</sup>, Mls<sup>b</sup>. Recipients were lethally irradiated (850 rad) before reconstitution. At different intervals after reconstitution a number of spleen cells equivalent to one spleen was transferred i.v. into secondary recipients which were syngeneic to the original spleen cell donors. Peak one-way MLC responses are expressed as specific counts per min. Figures represent the arithmetic mean ± 1 s.e.m. of a quadruplicate microculture. The day of peak MLC response is given in parentheses. Each culture consisted of  $5 \times 10^5$  responder spleen cells and  $5 \times 10^5$  stimulator (treated with 25 µg mitomycin C per ml) spleen cells. Activity of responder cells alone was 250–1,200 c.p.m. and activity of mitomycin C-treated stimulator cells 50–300 c.p.m. Background activity was 35 c.p.m. Details of the technique will be published elsewhere<sup>15</sup>. Challenge was carried out as described in Table 1 legend. Peak DTH responses are expressed as the specific percentage increase in foot thickness. Figures represent the arithmetic mean ± 1 s.e.m. of five mice. The day of peak DTH response is given in parentheses.

**Table 3** Anti-graft immune reactivity after immunisation with spleen cells differing at H-2 subregions or minor histocompatibility loci

Immunisation	Responder	Challenge	H-2 subregion or Mls locus-coded antigens of cells used for challenge	DTH Response
B10.AQR	B10.A	B10.AQR	K	39.2 ± 2.8
B10.AQR	B10.T(6R)	B10.AQR	I	38.0 ± 3.2
A.TL	A.TH	A.TL	I(+Tla)	33.6 ± 3.0
A.SW	A.TH	A.SW	D(+Tla)	26.6 ± 2.3
BALB/c × DBA/2	BALB/c	DBA/2	Mls <sup>a</sup>	39.2 ± 2.2
BALB/c × DBA/2	BALB/c	B10.D2	Mls <sup>b</sup>	28.4 ± 3.0
BALB/c × DBA/2	DBA/2	BALB/c	Mls <sup>b</sup>	44.4 ± 2.8
AKR/FuRdA	C3H/f	AKR/FuRdA	Mls <sup>a</sup>	31.0 ± 2.2
AKR/FuRdA	C3H/f	B10.BR	Mls <sup>b</sup>	22.1 ± 2.0
C3H/f	AKR/FuRdA	C3H/f	Mls <sup>c</sup>	34.6 ± 2.4
C3H/f	AKR/FuRdA	B10.BR	Mls <sup>b</sup>	28.8 ± 1.5

Immunisation was carried out with  $1 \times 10^7$  spleen cells s.c., equally distributed over the inguinal areas by means of a 28-gauge needle. For the origin of H-2 subregions and Mls locus see legends to Tables 1 and 2. Challenge was carried out as described in the Table 1 legend. DTH responses were assayed 5 d after s.c. immunisation of the responder mice and expressed as the specific percentage increase in foot thickness. Figures represent the arithmetic mean  $\pm$  1 s.e.m. of five mice.

H-2 subregion-coded antigens and minor histocompatibility antigens to induce such T-effector cells after (semi-)allogeneic spleen cell transplantation in lethally irradiated mice. These results were correlated with the capacity of the same antigens to induce DTH-related T-effector cells during a HvG reaction.

In lethally irradiated congenic mice, reconstituted with allogeneic spleen cells which were different at the whole or part of the H-2 complex, it could be demonstrated that the transferable anti-host DTH reactivity is directed exclusively to the I region of the H-2 complex; K and D subregion-coded antigens do not induce anti-host DTH T-effector cells (Table 1). H-2 compatible, non-H-2-incompatible spleen cell transplantation in lethally irradiated hosts revealed that antigens encoded by the Mls locus can also elicit the development of a transferable anti-host-directed DTH reactivity (Table 2). Mls<sup>a</sup> and Mls<sup>c</sup> coded antigens initiated both a positive MLC response and a distinct GvH-related DTH reactivity. Mls<sup>b</sup>-locus antigens, on the other hand, were not able to initiate *in vitro* proliferation; this was associated with a marginal and short-lasting GvH-related DTH reactivity (Table 2).

Minor histocompatibility antigens other than Mls-locus products did not contribute to the expression of the anti-host DTH reactivity. This was shown by B10.D2 challenge of secondary BALB/c recipients of cells activated to DBA/2 non-H-2 antigens, and by B10.BR challenge of C3H/f and AKR recipients of cells activated to AKR and C3H/f, respectively. In this experiment the cells used for challenge had different Mls-locus antigens from the irradiated recipients. As there is extensive cross-reactivity in various mouse strains between non-H-2 alloantigens other than Mls-locus products<sup>11</sup>, challenge with H-2-compatible, Mls locus-different spleen cells would demonstrate the contribution of these non-H-2 alloantigens in the expression of GvH-related DTH reactivity. No significant anti-host DTH reactivity could be elicited after challenge with B10.D2 or B10.BR spleen cells (Table 2). Therefore, non-H-2 alloantigens other than Mls locus-coded products probably do not evoke anti-host DTH reactivity. Apparently, Mls-locus products and I-region antigens have a similar capacity for inducing anti-host-directed DTH T-effector cells. K and D region antigens and non-H-2 antigens other than Mls-locus products are much less able to do so.

In contrast, in HvG reactions induced by subcutaneous (s.c.) injection of allogeneic spleen cells, K, D and I region-coded antigens as well as non-H-2 histocompatibility antigens could give rise to anti-graft DTH T-effector cells (Table 3). Also, minor histocompatibility antigens other than Mls-locus products contribute to the expression of the anti-graft DTH reactivity (Table 3). According to the work of Vadas *et al.*<sup>12</sup> there may be two subsets of T cells mediating DTH: Lyt 1<sup>+</sup> and Lyt 2<sup>+</sup> T cells, which are restricted by the I or the K and D regions of the MHC, respectively. Probably, in GvH conditions only Lyt 1<sup>+</sup> DTH

T-effector cells are activated (anti-H-2I and anti-Mls response) and in HvG conditions both Lyt 1<sup>+</sup> and Lyt 2<sup>+</sup> DTH T-effector cells are activated. This might be related to the frequency of T cells reactive to the various histocompatibility antigens<sup>13,14</sup>.

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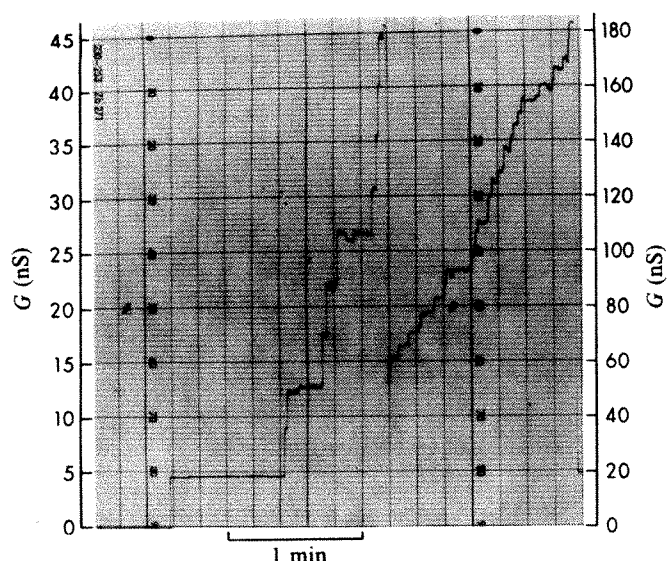
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## A candidate for the permeability pathway of the outer mitochondrial membrane

UNLIKE most other membranes, the outer mitochondrial membrane seems to be freely permeable to various small molecules and has therefore been called 'leaky'. The intactness of this membrane is demonstrated by observations<sup>1-4</sup> which show it to be impermeable to large polymers. This 'sieving' property is consistent with the presence of size-selecting units such as channels. Parsons and co-workers<sup>5,6</sup> have published electron micrographs showing what looked like channels in the outer membrane of mitochondria (particularly those from plants), and recent X-ray diffraction studies by Mannella and Bonner<sup>7</sup> have confirmed the existence of structures of similar dimensions to those seen in the electron micrographs. I now report the extraction of channel-forming material from the outer membrane of rat liver mitochondria. This material has properties which are





**Fig. 1** Incorporation of Triton X-100-solubilised VDAC into a planar bilayer. An unmodified planar lipid bilayer was made (see Table 1 legend) with a conductance of  $\sim 10$  pS (membrane was 0.15 mm in diameter) in the presence of 1 M KCl, 5 mM CaCl<sub>2</sub>. Five microlitres of a 2% Triton solution containing VDAC from rat liver mitochondria (a purified fraction—the method of purification will be described elsewhere) was added to the front compartment. A transmembrane voltage of 10 mV (the rear compartment being positive) was applied continuously using the voltage clamp set-up previously described<sup>8</sup>. After a few minutes, the insertion of channels is observed as distinct increases in membrane conductance. The channels are of uniform size. In the middle of the record the scale was reduced by a factor of 4, so the scale on the right-hand side of the graph refers to the right-hand side of the record while that on the left-hand side refers to the left side of the record. The chart speed was constant throughout.

essentially identical with those reported previously<sup>8</sup> for voltage-dependent anion-selective channels (VDAC) from mitochondria of *Paramecium*. VDAC could be responsible for the permeability of the outer membrane to small molecules and may be involved in regulating mitochondrial metabolism.

The VDAC channels previously described (from *Paramecium* mitochondria) displayed the following properties after insertion into planar lipid bilayers: (1) The permeability produced in the bilayer is due to channels of uniform size. (2) These channels conduct anions better than cations of comparable size and charge. (3) The channels switch to lower conducting states (close) when the transmembrane voltage is increased both in the positive and negative direction: the voltage range at which this switching from high to low conductance occurs is narrow so that the voltage dependence is steep. These properties characterise VDAC and distinguish it from all other conductances that have been observed in bilayers. These properties are constant from preparation to preparation and, remarkably, from species to species. Hence, the term VDAC is used here to refer to all the channels displaying these characteristic properties.

Channel-forming activity was assayed in two ways as described in Table 1 legend and conductance in the bilayer was checked for the characteristic properties described above. In the first assay method the sample (suspended in hexane) was layered on the surface of the aqueous phases to generate monolayers from which the bilayer was formed (no detergent was used). The numbers of VDAC channels in the resulting bilayer are a measure of VDAC activity. Results obtained with this method are shown in the third column of Table 1. The second method involved extracting a sample with Triton X-100 and adding an aliquot of this extract to the medium bathing an unmodified lipid bilayer. The rate of VDAC channel insertion from the medium per unit time is a measure of the VDAC content of the original sample (last column in Table 1). Both methods yield VDAC channels with the same characteristic properties. Therefore the presence of the detergent has no discernible effect on VDAC.

Table 1 shows that mitochondria from a wide variety of eukaryotes have VDAC channels. To date all mitochondria tested have shown VDAC activity. In addition, Table 1 shows that VDAC is not present in rat liver microsomes. The low activity in the microsomal fraction can be accounted for by a 1% mitochondrial contamination. The large standard deviations shown in the table do not indicate irreproducibility in obtaining VDAC activity. Rather, there was a large variation in the number of channels incorporated in the bilayer using the same preparation on the same day. (This type of variability is not unusual when conducting elements are inserted into planar lipid bilayers.) Despite quantitative uncertainty, there is no doubt that VDAC is present in all these organisms.

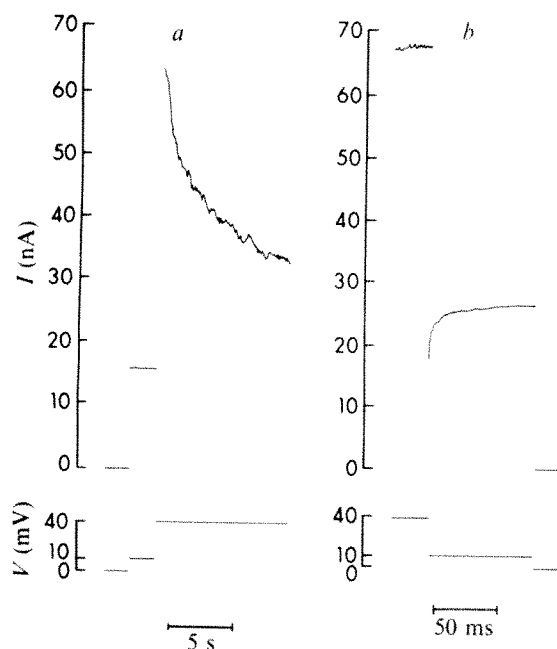
Figure 1 shows a time course of the current (number of ions crossing the membrane per unit time) which flowed across the membrane after the addition of an extract of rat liver mitochondria to the aqueous phase in which the membrane was bathed. The driving force for ion flow was a 10-mV transmembrane potential. The current increased by distinct jumps of uniform size. The rise time of the jumps was shorter than we can accurately measure,  $< 50$   $\mu$ s—a characteristic property of channels in general. The rate of channel insertion was fairly constant.

The voltage dependence of VDAC is illustrated in Fig. 2. When the driving force was increased from 10 to 40 mV the current increased fourfold but then decreased to a new lower level. This was due to the switching of VDAC channels from a high to a low conductance state (channels close). When the voltage was returned to 10 mV the channels returned to the high conductance state (channels open). Note that the rate for VDAC opening is much faster than that for closing. The same behaviour

**Table 1** Location of VDAC at the organelle level

Source	Major organelle present in fraction	VDAC activity	
		Channels per membrane	Channels per min per mg*
<i>Paramecium</i>	Mitochondria	500 $\pm$ 300	
	Cilia	0	
	Pellicle	20 $\pm$ 20	
Rat liver	Mitochondria	200 $\pm$ 100	
	Microsomes	2 $\pm$ 1	0
Rat heart	Mitochondria		330
Beef heart	Mitochondria		370
<i>Neurospora</i>	Mitochondria	700 $\pm$ 600	1,000 $\pm$ 500
Yeast	Mitochondria	50 $\pm$ 50	
Lobster	Axolemma	0	0

Mitochondrial fractions from rat, beef, *Neurospora* (wall-less mutant of *N. crassa*, provided by Dr Gene Scarborough) and yeast were produced by standard procedures. The rat liver microsomes were obtained by centrifuging the post-mitochondrial supernatant at 100,000g for 30 min and taking the top part of the translucent pellet. The *Paramecium* results are those reported previously<sup>8</sup>. Purified lobster axolemma was prepared as described by Barnola *et al.*<sup>16</sup>. Channel-forming activity was assayed by two methods. The first was that described previously<sup>8</sup>. [Mitochondria were sonicated with a 25-fold excess of soybean phospholipid, lyophilised and suspended in hexane (1% w/v). The suspension was layered on the aqueous phases to form monolayers which were then used to produce bilayers by the method of Montal and Mueller<sup>17</sup>.] Results of this assay are expressed as channels per membrane  $\pm$  s.d. In the second method, Triton X-100 was added to the sample to a final concentration of 2% (v/v) (the protein concentration was not greater than 10 mg ml<sup>-1</sup>). A planar bilayer was made by the method of Montal and Mueller<sup>17</sup> following the technique previously described<sup>8</sup>. (Soybean lipids were used after purification as previously described<sup>18</sup>.) An aliquot of this solution (5 or 10  $\mu$ l) was added to the front compartment. The rate of incorporation of channels into the bilayer, normalised per unit amount of protein added, is a measure of the VDAC activity in the sample (last column). (Note that the Triton extract should be warmed up to room temperature for at least 1 h before assay.) Normalisation is not required in the first assay because the ratio of protein to lipid in the membrane forming solution is kept at a constant 1:25 (w/w). \* Rate of VDAC channel insertion from medium per unit time per mg protein.



**Fig. 2** The voltage-dependent conductance of VDAC. The upper traces show the current and the lower traces the voltage as a function of time. VDAC was inserted as described in Fig. 1. After a certain time the amount of VDAC inserting, for short time periods, is small compared with what has already inserted. Therefore, the conductance can be considered constant for short periods so that conductance changes caused by voltage are not due to channels inserting into the bilayer. On the left-hand graph (a), the voltage was increased from 0 to 10 to 40 mV. The current was constant at 0 and 10 mV. When the voltage was increased to 40 mV, initially, the current increased ohmically indicating no conductance change. Then the current decreased as the VDAC channels closed. On the right-hand graph (b), the membrane was clamped at 40 mV until the current level was fairly constant (channels had turned off). Then the voltage was dropped to 10 mV. The current initially dropped to  $\frac{1}{4}$  of its value (ohmic behaviour) and then increased due to VDAC channels opening. Note the difference in time scale between closing and opening rates. The records were first stored using a digital storage oscilloscope (Gould OS 4000) and then transcribed onto a chart recorder.

was seen in VDAC channels from *Paramecium* mitochondria<sup>8</sup>. This was also observed with VDAC channels from *Neurospora crassa* and *Saccharomyces cerevisiae*.

The anion selectivity was verified by measuring the reversal potential of the membrane conductance in the presence of a salt concentration gradient (KCl at 1.0 and 0.1 M). A value of 13 mV positive in the high salt side was measured for material extracted from rat liver—close to that observed in VDAC from *Paramecium* (Fig. 5b of ref. 8).

VDAC's large single channel conductance (0.45 nS in 0.1 M KCl and 4.5 nS in 1.0 M KCl) indicates that the pore size is large. A simplified calculation assuming the specific conductance in the channel to be the same as in solution yields a pore diameter of  $\sim 15$  Å. Large pore size is also suggested by the fact that acetate<sup>8</sup>, sulphate<sup>8</sup>, fumarate and succinate (unpublished observations) easily cross the channel. (Fumarate is five times less permeable than  $\text{Na}^+$ .)

VDAC was shown to be located in the outer membrane of rat liver mitochondria by fractionating the mitochondrial membranes into outer and inner (fractions B and P) by the method of Parsons *et al.*<sup>9</sup>. Cross contamination was monitored using cytochrome oxidase activity<sup>10</sup> as a measure of the amount of inner membrane present in the outer membrane fraction and NADH-cytochrome *c* reductase activity<sup>11</sup> as a marker for outer membrane. VDAC activity was assayed by following the rate of channel insertion into a lipid bilayer as described above. The results are summarised in Table 2. The marker enzymes confirm good purification of inner and outer membranes with little cross-contamination. VDAC activity is sixfold higher in the

**Table 2** Location of VDAC in the mitochondrion

	Inner membrane fraction	Outer membrane fraction	Activity ratio outer/inner
Cytochrome oxidase ( $\mu\text{mol}$ cytochrome <i>c</i> $\text{mg}^{-1} \text{min}^{-1}$ )	1.04	0.063	0.06
NADH-cytochrome <i>c</i> reductase ( $\mu\text{mol}$ cytochrome <i>c</i> $\text{mg}^{-1} \text{min}^{-1}$ )	0.24	6.0	25
VDAC (channels $\text{min}^{-1} \text{mg}^{-1}$ )	$200 \pm 200(7)^*$	$1,200 \pm 1,000(6)$	6

\* Mean  $\pm$  s.d. with number of assays in parentheses.

outer membrane fraction than in the inner membrane. The large standard deviation is again due to the variability inherent in the assay method. Despite this variability, VDAC is clearly located in the outer mitochondrial membrane. (Its absence from the inner membrane has not been demonstrated.)

Thus the large size of VDAC, its location in the outer mitochondrial membrane and its presence in a wide variety of organisms, indicate that VDAC may be the channel making the outer membrane permeable to small molecules. It is conceivable that the voltage-dependence of VDAC may regulate the flow of metabolites between the cytoplasm and the inner mitochondrial membrane. The small transmembrane potential needed to turn off most of the channels could be generated by a Donnan potential, changes in surface or dipole potentials mediated perhaps by changes in enzyme activity or metabolite concentration.

Interestingly, a similar channel has been isolated from the outer membrane of *Escherichia coli*<sup>12,13</sup>. Genetic studies<sup>14</sup> and transport measurements of the reconstituted material in vesicles<sup>15</sup> show that there is a channel in the outer membrane of *E. coli* that allows small molecules up to the size of a triose to cross that outer membrane. Benz *et al.*<sup>12</sup> and Schindler *et al.*<sup>13</sup> have incorporated this protein into planar lipid bilayers and shown that it forms channels that are somewhat smaller than VDAC, weakly selective for cations over anions and voltage dependent, in a similar way to VDAC, but at high transmembrane voltages (150–200 mV compared with 15–30 mV for VDAC). This parallelism may indicate a common primordial channel.

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## A stable chemiluminescent-labelled antibody for immunological assays

RADIOISOTOPES, in particular  $^{125}\text{I}$ , have been used for many years as labels for monitoring the distribution of reagents in immunological assay systems. Satisfactory labelled derivatives are not always easily produced as iodination frequently produces severe molecular disruption. Moreover, iodinated proteins have a limited shelf life and sometimes require extended counting times for accurate quantitation. Recently, non-radioactive labels such as bacteriophages<sup>1,2</sup>, enzymes<sup>3,4</sup>, stable free radicals<sup>5</sup> and fluorescent groups<sup>6</sup> have been used in attempts to overcome some of these problems. We describe here the successful labelling of antibodies to rabbit IgG with a chemiluminescent molecule and the development of an immunological assay using chemiluminescence as a means of monitoring reagent distribution.

The luminescence of luminol (5-amino-2,3-dihydrophthalazin-1,4-dione) occurs during oxidation in alkaline conditions<sup>7</sup> in the presence of certain inorganic ions or molecules<sup>8</sup> (for example  $\text{MnO}_4^-$ ,  $\text{I}_2$ ,  $\text{Fe}(\text{CN})_6^{3-}$ ,  $\text{Cu}^{2+}$  and  $\text{OCl}^-$ ). In such conditions, luminol takes part in a reaction which generates an excited product molecule. This subsequently becomes de-excited by several mechanisms<sup>9</sup>, one of which is the emission of photons. The quantum yield for this process is approximately 0.015 in aqueous conditions<sup>10</sup>. A photon counter built in our laboratory<sup>11</sup> is capable of detecting  $10^{-18}$  mol of luminol.

An IgG fraction of a sheep anti-rabbit IgG antiserum prepared by sodium sulphate precipitation was reacted with a diazonium salt of luminol. Diazotisation involved a marked reduction in quantum yield (Table 1), possibly as a result of polymerisation of diazoluminol. A further small reduction took place following overnight incubation at pH 8.6 as required for the coupling reaction, although there was no further loss of quantum yield as a result of the coupling procedure itself. The incorporation of diazoluminol into conjugate with IgG was dependent on the concentration of reactants (Table 2). Using a 100-fold molar excess of luminol it was possible to incorporate up to 80% of the ligand, although it was found that at uptake

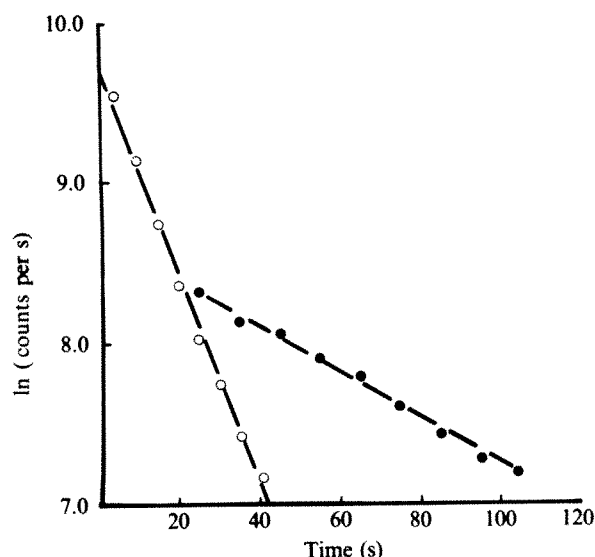
**Table 1** Recovery of luminescence during coupling of luminol to IgG

Stage of coupling	Total recovered counts	% Initial luminescence
Initial	$5.9 \times 10^{11}$	100
Diazoluminol*	$7.9 \times 10^9$	1.34
Diazoluminol†	$3.8 \times 10^9$	0.67
Lumino-IgG conjugate	$4.0 \times 10^9$	0.70

Luminol-IgG conjugate was prepared by diazotising 200 mg of luminol in 20 ml ice-cold 2.4 M HCl with 200 mg  $\text{NaNO}_2$ . Ice-cold borate buffer (50 ml 0.5 M, pH 8.5) was added and the pH adjusted to 8.5. Fourteen ml of this solution was added to 8.5 ml of a solution containing 1 g of an IgG fraction, prepared by sodium sulphate precipitation of a sheep anti-rabbit IgG antiserum (code SB 82/2) in borate buffer (0.5 M, pH 8.5). The molar ratio of luminol: IgG was calculated to be approximately 30:1. The mixture was allowed to stand for 18 h at 4 °C to enable coupling to occur. Unconjugated luminol was removed by extensive dialysis against 0.2 M borate buffer pH 8.0 and stored as 0.5 ml aliquots at -20 °C. Samples (10  $\mu\text{l}$ ) were removed at stages during the preparation of the conjugate and diluted (1/100–1/1,000) with 0.1 M NaOH. Luminescence was measured as follows. Aliquots (10  $\mu\text{l}$ ) of the diluted samples were added to 1 ml of 0.1 M NaOH in polystyrene tubes. 5.4 mM  $\text{H}_2\text{O}_2$  (100  $\mu\text{l}$ ) was added and after mixing the reaction tube was placed in the reaction chamber of the digital photon counter. The reaction was initiated by addition of 1 ml 1.0 mM NaOCl in 0.1 M NaOH solution. Counts were accumulated for a period of 20 s. After correction for volume changes values were calculated to give total recovered counts.

\* Immediately on preparation.

† After overnight incubation at 4 °C in 0.2 M borate buffer (pH 8.5).



**Fig. 1** Time course of light emission from luminol (●) and luminol-IgG conjugate (○). The luminol-IgG conjugate was prepared as described in Table 1, and purified before use by affinity chromatography using an immunoabsorbent (ImAd) prepared from rabbit IgG coupled to a diazonium salt of powdered cellulose<sup>12</sup>. After reaction overnight at 4 °C the ImAd-antibody complex was washed free of unbound label using 0.05 M veronal buffer, pH 8.2 containing (w/v) 0.1% bovine serum albumin, 0.05% sodium azide 0.002% non-immune sheep IgG (NISH buffer). After washing with water to remove the buffer, luminol-labelled antibody was eluted by reduction of the pH to 2.0. The supernatant was buffered to pH 8.2 using an equal volume of NISH buffer at double strength with respect to all constituents. The luminescence was quantitated as described in Table 1. In this case the rate of light emission was plotted on a chart recorder. Values taken from this graph were plotted as the natural logarithm against time (in seconds) from the start of the reaction.

ratios in excess of 40 mol luminol per mol IgG the product precipitated on storage. The labelled product was freed of unreacted luminol by extensive dialysis and purified subsequently by affinity chromatography using a rabbit IgG immunoabsorbent. The purified product prepared by reacting IgG of the sheep anti-rabbit IgG antiserum with a 30-fold molar excess of diazoluminol contained 22 mol luminol per mol IgG.

Figure 1 compares the time courses of light emission from luminol and the luminol-antibody conjugate. The two materials were tested in identical conditions as the rate constant for light emission is dependent on the concentration of  $\text{H}_2\text{O}_2$  and catalyst<sup>8</sup>. Luminol conjugated to IgG had a significantly higher rate constant than luminol itself. When the luminescent material was the limiting reactant, the decay phase of the light-emitting reaction was an exponential curve with a rate constant of  $0.073 \text{ s}^{-1}$  for conjugated luminol and a value of  $0.014 \text{ s}^{-1}$  for luminol itself. The reason for the increase in reaction rate of conjugated luminol is not yet clear. It is possible that some structural alteration in the ligand has taken place.

In these experiments we have quantified the luminescent material by measuring accumulation of counts during a 20-s period. It is apparent from Fig. 1, however, that in the conditions used for these experiments, the rate of emission of light from the reaction is first order with respect to the luminol concentration. It is therefore possible to quantify the luminescent material by measuring the maximum reaction rate over a short period, for example 2 s.

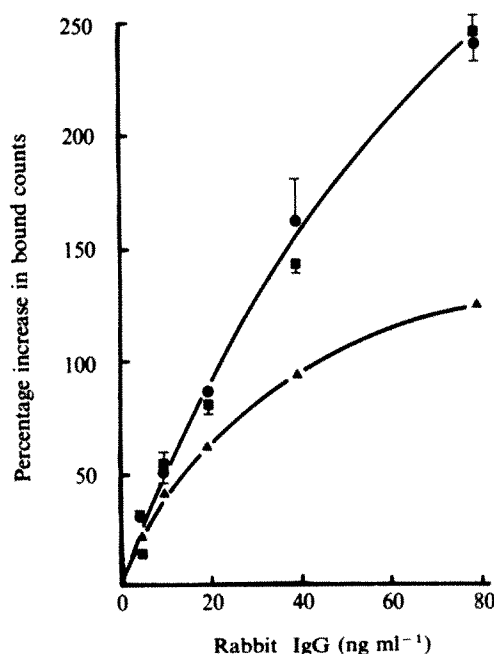
The immunological reactivity of the luminol-labelled sheep anti-rabbit IgG antibody was assessed by a radioimmunoassay using solid phase rabbit IgG and  $^{125}\text{I}$ -labelled sheep anti-rabbit IgG antibody. Through a series of dilutions (1/1 to 1/1,000 with respect to the antiserum) no difference could be detected between the luminol-labelled antibody and unlabelled sheep anti-rabbit IgG antibody in ability to bind antigen.



Using the luminol-labelled sheep anti-rabbit IgG antibodies it was possible to develop a two-site assay<sup>13</sup> for rabbit IgG. The procedure involved extraction of rabbit IgG on to polystyrene tubes coated with unlabelled sheep anti-rabbit IgG antibody. Uptake of the antigen was then determined by reacting with the labelled antibodies. Figure 2 shows standard curves obtained using luminol-labelled antibodies immediately after preparation (a) and after 9 months storage at  $-20^{\circ}\text{C}$  (b) with a curve obtained with freshly prepared  $^{125}\text{I}$ -labelled sheep anti-rabbit IgG antibody. The luminol-labelled antibody produced an assay of similar sensitivity to that obtained with the iodinated antibody but also showed no apparent loss of activity after storage for 9 months.

These results show that antibody molecules may be labelled with luminol without altering their immunological reactivity, and, despite losses in the quantum yield from the luminescent molecule, these antibodies may be used to develop a satisfactory immunological assay. Moreover, the time course of light emission from the labelled derivative is rapid enough for accurate measurement to be made within 2 s of the initiation of the luminescent reaction. Finally, the stability of the labelled antibody is such that assays may be carried out over long periods (at least 9 months) with no apparent loss of performance.

The development of chemiluminescent labels offers considerable potential for improvements in immunoassay technology and in other areas of biochemistry and histology where there is a need for biologically active high specific activity labels. Because they are stable, such reagents can be produced in bulk. This has important implications in terms of cost and quality control of assays. The ability to carry out extremely rapid



**Fig. 2** Standard curves obtained in a two-site assay of rabbit IgG using luminol-labelled and  $^{125}\text{I}$ -labelled (▲) sheep anti-rabbit IgG antibody. Assays were carried out with freshly prepared luminol-labelled antibody (●) and the same reagent after nine months storage at  $-20^{\circ}\text{C}$  (■). Antibodies purified from antiserum SB 82/2 by means of affinity chromatography with rabbit IgG ImAd, were incubated in polystyrene tubes in 0.1 M carbonate buffer pH 9.6 at a dilution equivalent to 1:85 of the original serum, to prepare a solid phase derivative<sup>14</sup>. After coating, the tubes were washed with 0.1% bovine serum albumin in 0.15 M sodium chloride solution containing 0.1% thiomersal, and stored empty at  $-20^{\circ}\text{C}$ . In the assay procedure, standard solutions of rabbit IgG in NISH buffer (half strength) were added to the tubes and incubated overnight at  $4^{\circ}\text{C}$ . Then, either luminol or  $^{125}\text{I}$ -labelled antibodies purified as described in Fig. 1 were added. After a further overnight incubation at  $4^{\circ}\text{C}$ , the tubes were washed again in NISH buffer (half strength) and either counted in an automatic gamma spectrometer or assayed for luminescence activity as described in Table 1.

**Table 2** Incorporation of luminol into luminol-IgG conjugate

Initial luminol/IgG ratio	$A_{450}^a$	$A_{450}^b$	TCA precipitated <sup>c</sup> (%)	Molar ratio of luminol: IgG <sup>d</sup>
0	0.000	0.000		
5	0.172	0.098	43.0	2.15
10	0.199	0.067	66.3	6.63
20	0.228	0.074	67.5	13.5
50	0.419	0.077	81.6	40.8
100	0.758	0.127	83.25	83.25

Luminol and diazoluminol are both soluble in TCA solutions. Both compounds absorb strongly at 450 nm.

<sup>a</sup> Absorbance (450 nm) of conjugate after coupling, before dialysis.

<sup>b</sup> Absorbance (450 nm) after precipitation of protein by addition of an equal volume of 15% trichloroacetic acid (TCA). The supernatant was buffered to pH 8.5 and a correction made for volume change.

<sup>c</sup>  $(1 - b/a) \times 100\%$ .

<sup>d</sup>  $c/100 \times \text{initial ratio}$ .

quantitation of label is likely to prove an important factor in the organisation of assays involving large sample numbers, for example screening programmes. Finally, the quantitation of chemiluminescence offers a major advantage, particularly with respect to sensitivity, over other non-isotopic labels (for example fluorescence) in that detection is made against a background which is theoretically zero.

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## Introns in the chicken ovalbumin gene prevent ovalbumin synthesis in *E. coli* K12

It has recently been discovered that some eukaryotic genes are split, the mRNA coding sequences (exons) being interrupted by intervening sequences (introns) of unknown function<sup>1-3</sup>. In all cases examined, these split genes are transcribed into an RNA precursor which is then matured by a splicing mechanism<sup>4-6</sup>. Because this type of gene structure has not previously been found in bacteria, it is generally believed that split genes cannot be expressed in *Escherichia*, but there are no facts to support this assumption. We demonstrate here that the presence of introns in the chicken ovalbumin gene prevents ovalbumin synthesis in *E. coli* K12.

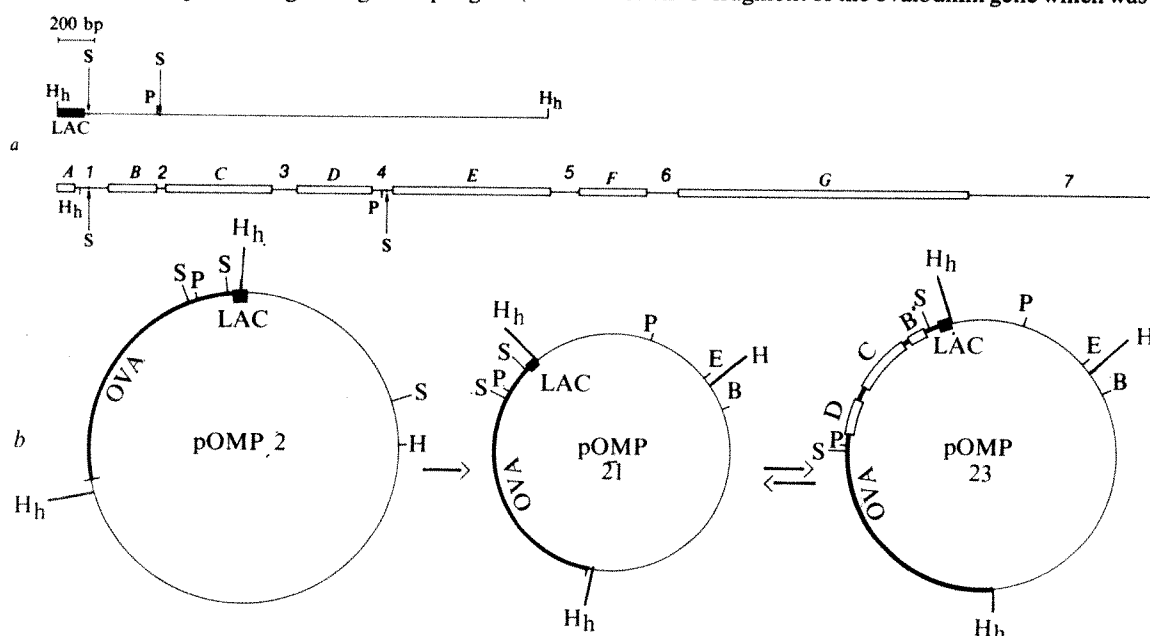
The chicken ovalbumin gene is split into eight exons (designated 1 to 8) separated by seven introns (named A to G)<sup>2,7-17</sup>. It

is transcribed into an RNA precursor (at least) 7,600 nucleotides long, which is then spliced into ovalbumin mRNA (ov mRNA)<sup>5,6</sup>. We have isolated, by molecular cloning in *E. coli* K12, chicken DNA segments which carry part of the gene or the entire gene<sup>7-14</sup>. We have also constructed plasmids which carry synthetic double-stranded DNA sequences complementary to ov mRNA (ov ds cDNA) and are devoid of introns<sup>17</sup>. In one of these plasmids, pOMP2, the ovalbumin sequence was fused in phase at the beginning of the *E. coli* gene coding for  $\beta$ -galactosidase, the fifth amino acid of ovalbumin (from the NH<sub>2</sub>-terminal part of the protein) being connected to the seventh amino acid of  $\beta$ -galactosidase<sup>18</sup>. We have shown (as have others<sup>19</sup>) that *E. coli* cells harbouring the recombinant plasmid synthesise large amounts of an ovalbumin-like product, which resembles ovalbumin in size and reactivity with ovalbumin-specific antibodies. We have manipulated the fused *lac*-ovalbumin sequence and introduced into it some of the introns present in the natural gene so as to assess their influence on the synthesis of the ovalbumin-like product.

There are two sites for the restriction endonuclease *Sst*I in ov ds cDNA, 378 base pairs apart, located at nucleotides 116 and 494 in the ov mRNA sequence<sup>8,11</sup>. In the natural gene, these sites are located in exons 1 and 4, respectively, and separated by 1,612 base pairs which include introns B, C and D (refs 8, 11). The substitution of this 1,612-base pair fragment in place of the 378-base pair one in the *lac*-ovalbumin sequence will thus result in the introduction of three introns of the natural gene. This was achieved as depicted in Fig. 1: because pOMP2 has an additional *Sst*I site (in a sequence originating from phage  $\lambda$  (ref.

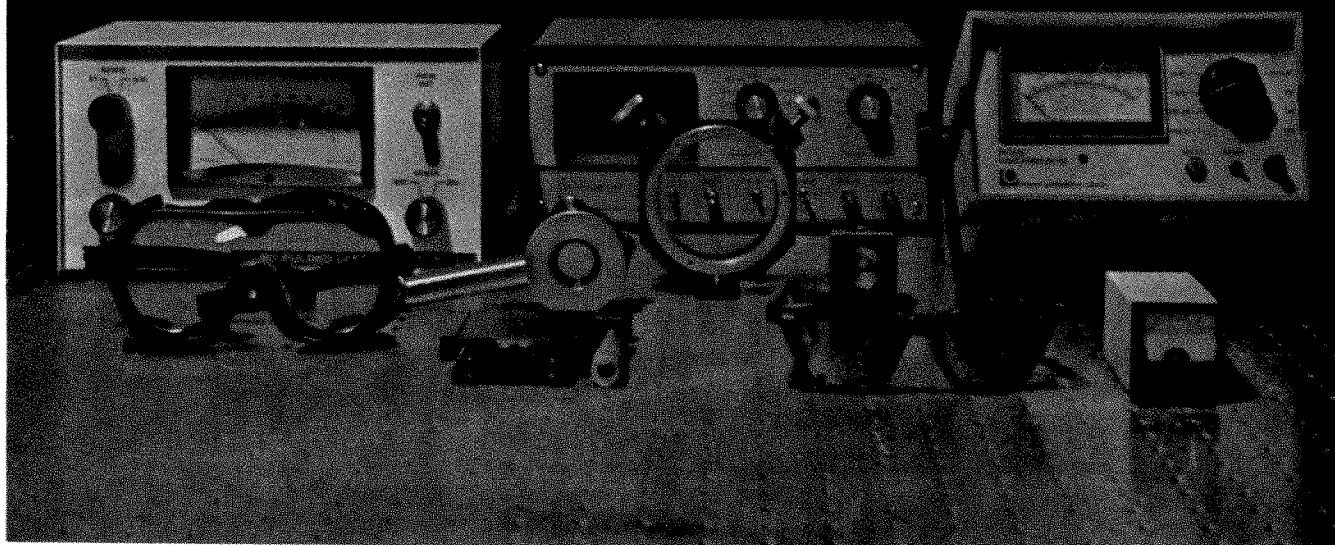
18)) we first extracted the *lac* regulatory region with the *lac*-ovalbumin fusion by digestion with *Hha*I. The resulting 2.67-kilobase fragment was introduced into partially digested pBR322, cut only once by *Hha*I. The resulting plasmids, like pOMP2, produced large amounts of ovalbumin-like protein in *E. coli*. One of these, pOMP21, was selected for further studies. The 378-base pair *Sst*I fragment of pOMP21 was substituted by the 1,612-base pair *Sst*I fragment extracted from the cloned *Eco*RI fragment 'b' of the ovalbumin gene<sup>8</sup>. We thus obtained pOMP23 in which introns B, C and D were correctly orientated for transcription from the *lac* promoter (see Fig. 1 legend for mapping of this plasmid). *E. coli* harbouring pOMP23 was then tested for the production of ovalbumin-like protein by a radioimmunoassay. As shown in Fig. 2, pOMP21 (as pOMP2) makes large amounts of an ovalbumin-like product, whereas no ovalbumin-like product is detectable in the pOMP23 assay, even with concentrated bacterial extracts—at least 3,000-fold less than pOMP21, that is, less than three molecules per bacterium in the experiment shown.

We next investigated whether this negative result could be due to inadvertent mutational changes during the construction of pOMP23. The *Sst*I 1,612-base pair fragment was excised from pOMP23 and replaced by the 378-base pair *Sst*I fragment extracted from ov ds cDNA. Where the insertion occurred in the proper direction, pOMP26 again produced large amounts of ovalbumin-like protein (not shown). This does not exclude the possibility that mutations occurred in the 1,612-base pair fragment. The latter, however, was isolated from the same cloned *Eco*RI 'b' fragment of the ovalbumin gene which was sequenced



**Fig. 1** Introduction of introns B, C and D into the *lac*-ovalbumin sequence. *a*, Restriction maps of the fused *lac*-ov ds cDNA sequence and of the ovalbumin *Eco*RI 'b' fragment (see also refs 11, 14, 15). The *Hha*I site in the *lac* regulatory region lies immediately after the TGA stop signal at the end of the *lac* I gene<sup>2</sup>. The *Hha*I site in exon 1 is the one used to construct the *lac*-ovalbumin in fused sequence in pOMP2 (refs 8, 18). *b*, Map of the plasmids pOMP2, pOMP21 and pOMP23, pOMP2 and its related plasmids have 32 *Hha*I sites<sup>21</sup>. Only those used to excise the *lac*-ovalbumin sequence are shown. The construction of pOMP23 involved the following steps: 20  $\mu$ g of pOMP2 DNA were digested to completion by *Hha*I (Biolabs; 25 units; 30 min at 37 °C) and the reaction was stopped by heating to 65 °C for 10 min in the presence of 10 mM EDTA. The reaction mixture was loaded on a 5–20% sucrose gradient (w/v in 25 mM Na acetate, pH 6.0, 10 mM EDTA, 0.5% SDS) and centrifuged at 31,000 r.p.m. for 16 h at 20 °C in a SW41 rotor of a Beckman ultracentrifuge. The 2.67-kilobase fragment carrying the fused *lac*-ovalbumin sequence could thus be purified from the other, much smaller fragments of pOMP2. pBR322 (ref. 21) DNA (20  $\mu$ g) was partially digested by *Hha*I. Enough enzyme was added to convert about half of the supercoils into linear molecules, which were purified by centrifugation through a 5–20% sucrose gradient (w/v in 10 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA; 4 h at 40,000 r.p.m. and 20 °C in a SW41 Beckman rotor). 200 ng of the 2.67-kilobase *lac*-ovalbumin *Hha*I fragment and 150 ng of linearised pBR322 were ligated with T4 DNA ligase at 4 °C in a final volume of 5  $\mu$ l in 40 mM Tris, pH 7.5, 10 mM MgCl<sub>2</sub>, 10 mM dithiothreitol, 0.1 mM ATP, 0.05 mg ml<sup>-1</sup> bovine serum albumin. After transformation of CaCl<sub>2</sub>-treated lysogenic cells of strain 1398 (ref. 22), transformants were selected on ampicillin-containing plates (20  $\mu$ g ml<sup>-1</sup>) and screened by *in situ* hybridisation<sup>23</sup> with a <sup>32</sup>P-labelled probe specific for the ovalbumin sequence (the *Hha*ov probe<sup>7,8</sup>). Positive colonies were then tested for ovalbumin production in the same radioimmunoassay as before<sup>18</sup>. All the clones positive in *in situ* hybridisation were also positive in this assay. Plasmid pOMP21 was arbitrarily chosen for further studies, and transferred into the non-lysogenic strain C600rk<sup>-</sup>mk<sup>+</sup>. pOMP21 (5  $\mu$ g) was then digested by *Sst*I (BRL, 2.5 h at 37 °C) and centrifuged in a 5–20% sucrose gradient (v/v in 10 mM Tris-HCl, pH 7.4; 20 mM NaCl; 1 mM EDTA; 45,000 r.p.m. for 140 min at 20 °C in a SW 50.1 Beckman rotor). The 6.65-kilobase fragment was treated with calf intestine alkaline phosphatase<sup>23</sup>; of this, 133 ng was ligated by T4 DNA ligase in a final volume of 5  $\mu$ l, with 100 ng of the 1.61-kilobase fragment released after digestion by *Sst*I of pBR322 carrying the *Eco*RI 'b' fragment of the ovalbumin gene. After transformation of C600rk<sup>-</sup>mk<sup>+</sup>, 12 ampicillin-resistant clones were analysed. Purified clear lysates were treated by *Hha*I and examined for the presence of the 3.9-kilobase fragment containing introns B, C and D. In such clones, the orientation of the integrated 1.61-kilobase fragment was determined by digestion with *Pst*I and *Hha*I. The unique *Pst*I site in the ovalbumin sequence lies at nucleotide 477 in exon 4 close to one end of the integrated fragment. In such analyses, plasmid pOMP23 yielded fragments of 2.13 and 1.78 kilobases (instead of 0.19 and 3.72 kilobases in the reverse orientation), demonstrating proper orientation of introns B, C and D. More precise mapping of plasmids pOMP21 and pOMP23 was achieved by the use of *Pst*I, *Bam*HI and *Sac*I. It indicated that the *lac*-ovalbumin fused sequence was most probably integrated into the *Hha*I site located at nucleotide 2,351 of the pBR322 sequence<sup>24</sup>. Replacement of the 1,612-base pair fragment of pOMP23 by the 378-base pair ov ds cDNA segment was carried out by the same procedures as above, except that *Sac*I was used instead of its isoschizomer *Sst*I (ref. 25). The letters S, P, E, B, H and Hh refer to *Sst*I, *Pst*I, *Eco*RI, *Bam*HI, *Hind*III and *Hha*I sites, respectively.

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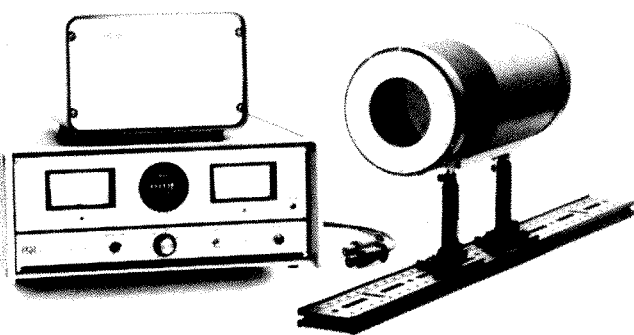
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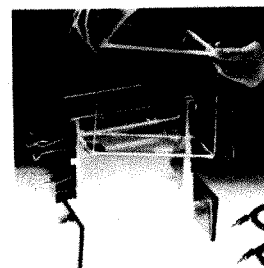
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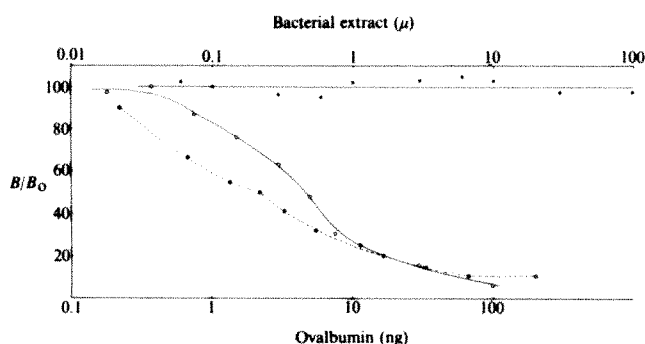
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**Fig. 2** Synthesis of ovalbumin-like protein by pOMP21 and pOMP23. C600rk<sup>+</sup>mk<sup>+</sup> (pOMP21) and C600rk<sup>+</sup>mk<sup>+</sup> (pOMP23) were grown overnight in L broth supplemented with 20  $\mu\text{g ml}^{-1}$  ampicillin, diluted into fresh medium containing 1 mM isopropylthiogalactoside, grown to about  $4 \times 10^8$  cells per ml, concentrated about 100-fold, and sonicated twice for 30 s as before<sup>18</sup>. The procedures for iodination of ovalbumin, purification of antibodies and radioimmunoassays have been described previously<sup>18</sup>. In the experiment shown, the dilution of antiserum chosen was able to precipitate 30% of the iodinated ovalbumin ( $B_0$ ). Results are expressed as  $B/B_0$ , the ratio between the c.p.m. precipitated in the presence or the absence of competing material (blanks being subtracted each time).  $B$  was measured in duplicate and  $B_0$  was the average of four determinations. ○, Results obtained with pure ovalbumin; ●, those obtained with pOMP21, representing the synthesis of about 6,500 molecules per bacterium. ●, Results obtained with pOMP23. Similar results are obtained in various hosts. C600rk<sup>+</sup>mk<sup>+</sup> carries the *supE* suppressor for amber mutations.

by K. O'Hare *et al.* (personal communication), and where the sequence of the various exons matched those of *ov* mRNA. It thus seems unlikely that inadvertent mutations cause the defect in expression of the ovalbumin-like product. Instead, we conclude that the introduction of introns *B*, *C* and *D* in the fused *lac*-ovalbumin sequence blocks the expression of ovalbumin-like product in *E. coli*.

The sequence data of K. O'Hare *et al.* indicate that introns *B* and *C* are 252 and 582 base pairs long (84 and 194 triplets, respectively) so that their presence will not alter the reading frame. In contrast, intron *D* consists of 401 base pairs (133 triplets plus 2 base pairs) and will cause a frameshift. (The sequence data of Robertson *et al.*<sup>28</sup>, obtained with another cloned DNA, show some differences; in their case, intron *C* would also cause a frameshift.) Further, intron *B* contains one nonsense codon (TGA), intron *C* shows 12 nonsense codons (four TAA, three TGA and five TAG) and intron *D* contains three TGA and three TAA stop signals. All these nonsense codons are in the same reading frame, and although the bacterial host used had some suppressing capabilities (see legend to Fig. 2) it is unlikely that efficient suppression could occur. Because, in addition, intron *D* causes a frameshift, any synthesis of ovalbumin-like product in *E. coli* would probably result from the splicing of intron sequences from the primary RNA transcript. The mechanisms involved in RNA splicing in the higher eukaryotes are still poorly understood. It is possible that additional, unknown eukaryotic genetic elements could promote splicing in *E. coli*. Also, we cannot rule out the possibility that the simultaneous presence of the seven introns of the ovalbumin gene is a prerequisite for splicing in *E. coli*. It seems very likely, however, that splicing does not readily occur in *E. coli*, and that the isolation of split genes or the production of the corresponding proteins cannot be based at this stage on their expression in *E. coli*.

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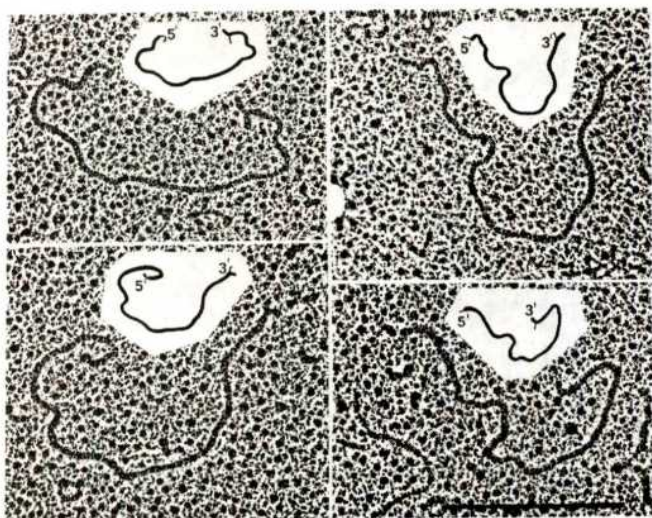
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## About 30% of minute virus of mice RNA is spliced out following polyadenylation

MANY viral and eukaryotic messenger RNAs have been shown to contain blocks of sequence which, although adjacent to one another in the RNA, are copied from non-contiguous regions of the genome that are joined together post-transcriptionally. This phenomenon of RNA splicing has been shown to occur during the processing of several DNA and RNA tumour viruses<sup>1-7</sup> and cellular<sup>8-10</sup> mRNAs. The mechanism by which splicing is carried out has not been elucidated, and any direct approach is complicated by the elaborate splicing patterns found in the systems which have been examined so far. We have extended these studies of RNA splicing to a potentially simpler viral system, the minute virus of mice (MVM), a non-defective member of the parvovirus group. The members of this group are considered to be among the simplest of the animal viruses, and comprise an icosahedral virion made from three structural protein species, containing one linear single-stranded DNA chromosome of  $\sim 1.5 \times 10^6$  molecular weight (for review see ref. 11). The MVM DNA molecule contains a stable hairpin duplex at its 5'-end and a 3'-terminal structure suitable for priming complementary strand synthesis *in vitro*<sup>12,13</sup>. Little is known about the transcription process in parvoviruses. The only cytoplasmic mRNA species found for adeno-associated virus (AAV), a member of the defective subgroup, is a transcript of 70% of the genome which has been shown by *in vitro* translation to code for the three capsid proteins<sup>14</sup>. The one study of transcription carried out in the non-defective subgroup has identified an analogous RNA species in Kilham rat virus (KRV)-infected cell cytoplasm which is complementary to 70% of the viral strand and sediments as a single component at about 18S (ref. 15). We report here our attempts to determine the sequence arrangement of MVM RNA by examining, in the electron microscope, hybrids formed between the abundant species of nuclear and cytoplasmic poly(A)<sup>+</sup> RNA and single-stranded genomic DNA. We have found that about 30% of MVM RNA is spliced out following polyadenylation.





**Fig. 1** Electron micrographs and their tracings of hybrids formed between nuclear poly(A)<sup>+</sup> RNA and MVM DNA. A-9 cells, a derivative of the mouse L-cell line ( $3 \times 10^7$  cells) were infected with 1–2 plaque-forming units per cell of plaque-purified strain T of MVM. At 20 h p.i., nuclear and cytoplasmic fractions were prepared<sup>18</sup>, and RNA extracted<sup>19</sup> and fractionated on oligo(dT) cellulose columns to poly(A)<sup>+</sup> and poly(A)<sup>−</sup> molecules. Genomic single-stranded DNA was extracted from purified virions<sup>12</sup>. The RNA was precipitated in ethanol with the genomic single-stranded DNA (0.5  $\mu$ g). The pellet was collected by centrifugation and resuspended in 66% (v/v) of repurified formamide, 3 M urea, 20 mM EDTA and 0.2 M Tricine-NaOH, pH 8.1. The hybridisation mixture was incubated at 37 °C for 6 h. Samples were prepared for visualisation in the electron microscope as detailed by Bratosin *et al.*<sup>22</sup>. Scale bar, 0.5  $\mu$ m.

Hybrids were obtained by annealing poly(A)<sup>+</sup> nuclear RNA and MVM genomic DNA. Figure 1 shows the appearance of representative RNA–DNA structures with their schematic tracings. Molecules of unit length MVM DNA were selected and examined for single- and double-stranded regions. Of more than 100 molecules scored, at least 95% exhibited double-stranded appearance over the entire length of the DNA. It should be emphasised, however, that the present analysis would not detect single-stranded regions of less than 50 nucleotides. Each hybrid examined shows a short Y-shaped structure at one end, consisting of one double-stranded and one single-stranded arm. The lengths of the two arms support the assumption that the double-stranded arm is the 130-base pair hairpin known to be at the 5'-end of the viral DNA<sup>12</sup> and that the single-stranded arm represents at least in part the poly(A) on the 3'-end of the RNA. The opposite end of these hybrid structures exhibit, in 50% of molecules examined, a short single-stranded region of up to 150 nucleotides, suggesting that the initiation of transcription might occur in a number of alternative sites. In this context it is noteworthy that SV40 nuclear and cytoplasmic RNAs have various 5'-terminal sequences<sup>20,21</sup>. As the complete (or almost complete) double-stranded hybrids formed with nuclear RNA are by far the most abundant structures observed in the electron microscope, it seems that poly(A)<sup>+</sup> nuclear RNA is a complete (or almost complete) transcript of the genomic DNA, and suggests that it is the precursor for cytoplasmic poly(A)<sup>+</sup> mRNA. Moreover, because the template for transcription is at least in part a linear DNA molecule (unpublished results), we conclude that transcription is always initiated within a region located at the extreme 3'-end of the viral chromosome. This correlates well with the proposed structure of the nuclear precursor for the AAV messenger RNA<sup>14</sup>.

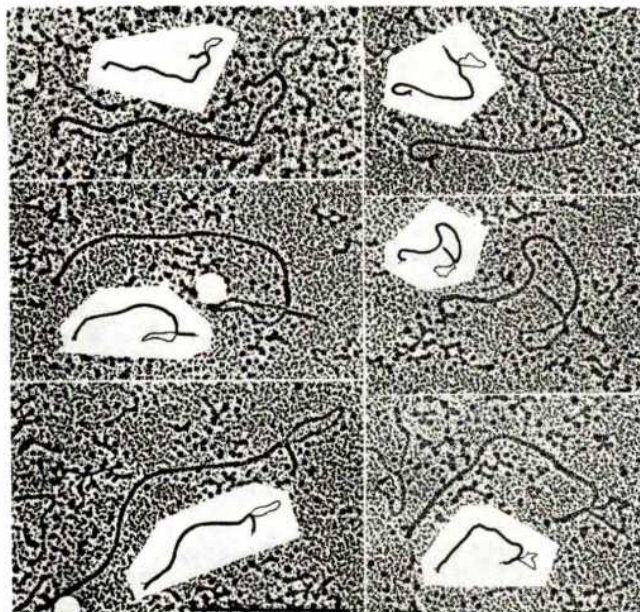
RNA was extracted from the cytoplasmic fraction of MVM-infected cells at 20 h post-infection (p.i.). Poly(A)<sup>+</sup> RNA was selected by chromatography on oligo(dT) cellulose columns. About 25% of <sup>3</sup>H-uridine-labelled poly(A)<sup>+</sup> cytoplasmic RNA (labelled 16–20 h p.i.) hybridised with genomic DNA, indicating

that the viral RNA comprises a substantial fraction of the poly(A)<sup>+</sup> RNA present in the infected cells.

Hybrids were obtained by annealing the poly(A)<sup>+</sup> cytoplasmic RNA and MVM genomic DNA. Figure 2 shows the appearance of representative RNA–DNA structures with their schematic tracings. As in the analysis of nuclear RNA, hybrids containing unit length MVM DNA were selected and examined for single- and double-stranded regions. Of about 100 molecules scored, more than 85% exhibited one short and one long arm of duplex with an intervening single-stranded DNA loop. As with the hybrids containing nuclear RNA, one end, that of the long duplex arm, has a short Y-structure comprising one double strand and one single strand, which we again postulate is the 3'-end of the RNA. As with the nuclear RNA–DNA hybrids, the opposite end, that of the shorter duplex arm, exhibited a short single-stranded region in many of the molecules examined. The observation made with the nuclear and cytoplasmic poly(A)<sup>+</sup> viral RNAs suggests that the MVM poly(A)<sup>+</sup> cytoplasmic RNA is a spliced molecule composed of two parts: a short 'leader' and a longer 'body'. The single-stranded DNA loop (see Fig. 2) contains the intervening template for RNA sequences which are present in the nuclear poly(A)<sup>+</sup> RNA and have been spliced out in the cytoplasmic poly(A)<sup>+</sup> RNA. The analysis of nuclear and cytoplasmic RNAs also indicates that the sequence of modifications during mRNA maturation is polyadenylation followed by splicing. We have measured the three parts on the RNA–DNA structures shown in Fig. 2 and the results of these measurements are presented in Fig. 3. Sharp histograms were obtained for each of the three parts: the leader spans  $8.8 \pm 1.2\%$  of the structure, the single-stranded DNA loop (or intron) spans  $29.3 \pm 2.4\%$  of the structure and the body spans  $61.8 \pm 2.3\%$  of the structure. The map coordinates of each part of the structures are represented in Fig. 3b.

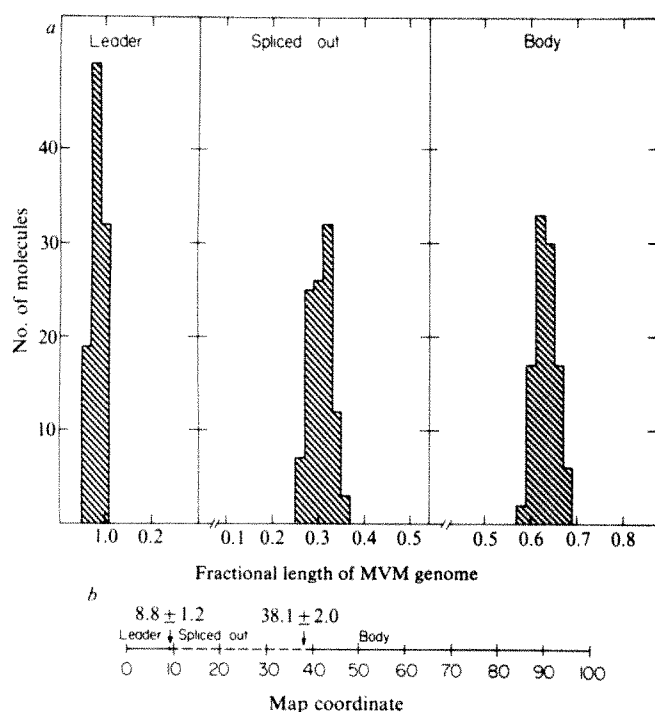
The present study does not indicate whether the poly(A)<sup>+</sup> cytoplasmic RNA which we have analysed is the only viral RNA present in the infected cells. However, if there are other RNA species coded for by the viral strand, its abundance should be less than 15% of this species. Similar analysis of poly(A)<sup>−</sup> RNA from infected cells revealed only a minor fraction of short heterogeneous RNA–DNA hybrids, with no accumulation of any particular length.

It is thought that the MVM genome has a small number of structural genes, perhaps only one<sup>16</sup>. The coding capacities of



**Fig. 2** Electron micrographs and their tracings of hybrids formed between cytoplasmic poly(A)<sup>+</sup> RNA and MVM DNA. Cytoplasmic poly(A)<sup>+</sup> RNA was prepared, annealed with genomic DNA and visualised in the electron microscope as detailed in Fig. 1. Scale bar, 0.5  $\mu$ m.





**Fig. 3** *a*, Histograms of the leader, intron and body obtained from analysing structures as shown in Fig. 2. One map unit (MU) corresponds to 1% of the length of the entire structure. *b* Shows the coordinates of each part on the viral DNA. The 3'- and 5'-ends of the genomic DNA were taken as 0 and 100 MU, respectively. Leader,  $8.8 \pm 1.2$  MU; spliced out,  $29.3 \pm 2.4$  MU; body,  $61.8 \pm 2.3$  MU.

the leader and body of the poly(A)<sup>+</sup> RNA are about 12,000 and 90,000 daltons, respectively. If only the body codes for polypeptides, as in adenovirus<sup>1</sup> and SV40 late mRNAs<sup>2</sup>, then it could code for polypeptide A (83,000 daltons) and protein B would be processed from this. If this is true then the viral mRNA would represent only about 70% of the viral genome and its primary transcript. Elucidation of the role of the remaining 30% of the primary transcript would be of great interest.

It is possible, however, that the MVM genome contains several genes, as a result of more than one functional initiation or termination site during translation. In this respect, it is noteworthy that splicing of RNA sequences at the post-transcriptional level could give rise to several functional mRNAs from the same precursor RNA, as has been found for SV40 early mRNAs<sup>17</sup>. These mRNAs could be translated in the same reading frame or switched from frame to frame.

The splicing of MVM RNA described in the present study resembles that of the mRNAs of other viruses and of eukaryotic cells but seems to be considerably less complex. Therefore, the transcription and processing of parvovirus mRNA provides a very useful model system for studying the mechanism of splicing and the physiological significance of this novel process in animal cells.

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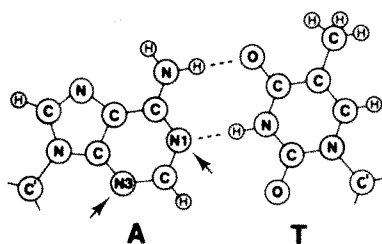
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## RNA polymerase unwinds an 11-base pair segment of a phage T7 promoter

WHEN RNA polymerase binds to a promoter site, it must unwind part of the DNA double helix so as to expose the template bases. Wang *et al.*<sup>1</sup>, Melnikova *et al.*<sup>2</sup> and Hsieh and Wang<sup>3</sup> have used different experimental techniques to measure the degree of unwinding. Their estimates range from 7 (ref. 1) to 15 (ref. 2) base pairs unwound per bound *Escherichia coli* RNA polymerase (EC 2.7.7.6). These studies, however, do not establish which part of a promoter is melted out. A new technique described here directly proves unwinding and furthermore identifies the exact region that RNA polymerase opens in the A3 promoter of phage T7. This promoter is one of three strong *E. coli* RNA polymerase promoters used early in the life cycle of phage T7 (ref. 4).

Dimethyl sulphate (DMS) methylates the N-7 position of G and the N-3 of A on double-stranded DNA<sup>5</sup>. On single-stranded DNA, however, the N-1 of A and to a much lesser extent, the N-3 of C can also be methylated<sup>5</sup>; these positions are not accessible on double-stranded DNA because they are involved in hydrogen bonding (Fig. 1).

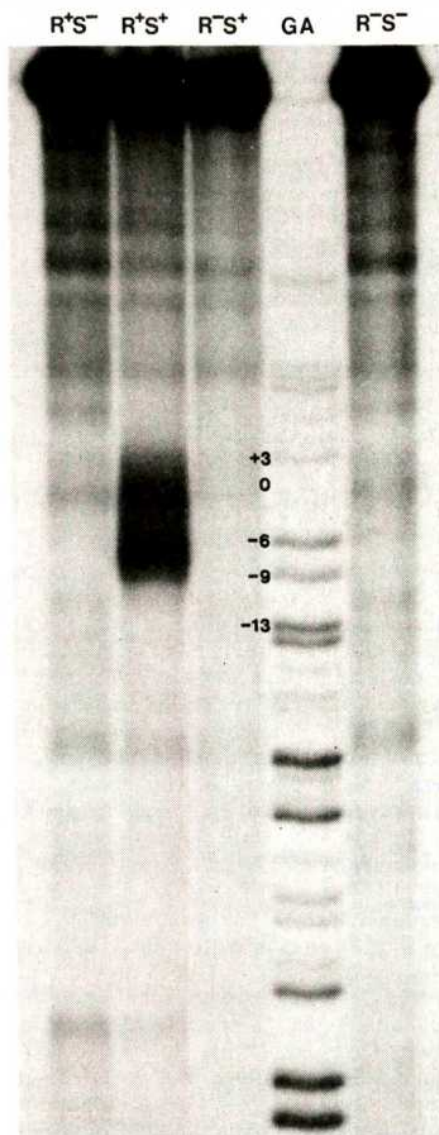
My technique takes advantage of this difference between single and double stranded DNA. First, I isolate the promoter on a restriction fragment which carries a <sup>32</sup>P label on the 5'-end of one strand only<sup>6</sup>. Then a complex between RNA polymerase holoenzyme and this promoter fragment is exposed to DMS. Wherever the RNA polymerase has unwound the double-stranded DNA, the N-1 position of A can be methylated. Precipitation of the DNA removes the RNA polymerase, allowing the two DNA strands to renature, except where the N-1 of A is methylated. The single strand-specific nuclease, S<sub>1</sub>, which cuts at unpaired bases<sup>7</sup>, will cut the DNA strand at mismatched A



**Fig. 1** AT base pair showing sites of methylation (see text).

and T bases created by methylation. This generates labelled strands, which terminate at the  $S_1$  cutting sites. When the  $S_1$  products are electrophoresed on a denaturing polyacrylamide gel<sup>6</sup> next to the sequence of the labelled strand, the lengths of the labelled  $S_1$ -cleaved strands identify the base pairs which were originally melted out by RNA polymerase.

Figure 2 shows an autoradiogram of such an experiment. The promoter fragment is an *HhaI*-*AluI* fragment of phage T7, carrying the A3 promoter, with transcription proceeding towards the *AluI* end<sup>4</sup>. As the *HhaI* end was labelled, Fig. 2 shows the  $S_1$  cuts on the sense strand. Due to the 3'-overhang at



**Fig. 2** Autoradiogram of a 12% polyacrylamide, 7 M urea gel<sup>6</sup> with the  $S_1$  nuclease digest of the A3 promoter fragment, which was previously methylated while complexed with RNA polymerase, in lane (R+S+). In the control lanes either  $S_1$  (R+S-) or RNA polymerase (R-S+) or both  $S_1$  and RNA polymerase (R-S-) were left out of the otherwise identical procedure. In another control, the DMS methylation was omitted from the protocol and again no  $S_1$  cleavage was observed (not shown here). The G/A lane represents the sequence of the fragment according to the 'G greater than A' method of Maxam and Gilbert<sup>6</sup>. The numbers indicate positions of the promoter sequence (see Fig. 3). A 0.5–1- $\mu$ g sample of the 155-base pair long *HhaI*-*AluI* fragment was preincubated with 9–10  $\mu$ g of RNA polymerase holoenzyme (greater than 90% saturation with the  $\sigma$  subunit) in 100  $\mu$ l of binding buffer (0.01 M  $MgCl_2$ , 0.1 mM EDTA, 1 mM dithiothreitol, 50 mM Na cacodylate pH 7.3, 0.05 M KCl) for 1–2 min at 37 °C. Then 3–4  $\mu$ l of a 10.4 M DMS solution were slowly added during the reaction, which was quenched (see ref. 6) after 3 min by adding 100  $\mu$ l of 1 M mercaptoethanol, 1% SDS and 2 M  $NH_4$  acetate, pH 5 (acidic conditions stabilise the labile N-1-methyl of A (ref. 5)). After precipitation with 2.5 volumes ethanol, the DNA was digested with approximately 4 units of  $S_1$  nuclease in 50  $\mu$ l of  $S_1$  buffer<sup>8</sup> (0.03 M Na acetate, pH 4.6, 0.05 M NaCl, 1 mM  $ZnSO_4$ , 5% glycerol) for 1 min at 37 °C. The reaction was stopped with EDTA and SDS.



**Fig. 3** A3 promoter sequence<sup>4</sup>. The canonical sequences<sup>4</sup> of the Pribnow box and the -35 region homologies, the initiation site and the unwound region are shown.

the *HhaI* site, the 5'-label here should not be accessible to  $S_1$  attack; nonetheless, about 25% of the counts cannot be precipitated after  $S_1$  incubation, which may reflect 'breathing' of the ends.

The experiment in Fig. 2 visualises the unwound region in lane (R+S+) and shows it to be an 11-base pair segment extending from about the middle of the Pribnow box, a region of strong promoter homology<sup>4</sup>, to just past the initiation site (Fig. 3). The same limits appear in the analogous experiment with the antisense strand (labelled at the *AluI* end) (data not shown). This proves that there was no significant nibbling back by  $S_1$  at the nicks, as that would shorten the size of the  $S_1$ -cleaved strands and, therefore, shift the limits for unwinding towards the 5'-labels of either strand. The blurring of the bands is probably due to extensive methylation which will insert extra positive charges.

$S_1$  cuts appear at almost every position of the unwound region (except around the two successive CG base pairs). Most probably, the limited  $S_1$  digestion causes cuts at either 5'- or 3'-side of a mismatched A or T, generating two labelled strand fragments which are separated by one nucleotide. This would account for  $S_1$ -cleaved strands migrating with Cs or Gs of the sequence; no significant methylation at the N-3 of C need have occurred. (Due to the 3'-OH end of  $S_1$ -cleaved strand fragments<sup>8</sup>, they will migrate slightly more slowly than the corresponding sequence strand fragment, which ends in 3'P (ref. 9).)

The unwound region I detect represents a minimum sequence, because if RNA polymerase were very tightly associated with the N-1 of an A: for example, it would prevent the methylation of that position and consequently the detection of such a melted base pair. Also, the CG base pairs bounding the unwound region may or may not have been opened.

The experiment directly shows that RNA polymerase unwinds a region covering 11 base pairs of the A3 promoter. This agrees well with previous estimates<sup>1-3</sup>. The unwound region includes the initiation site and, more surprisingly, the latter half of the Pribnow box homology, suggesting that the Pribnow box may serve to initiate unwinding. I have probed for specific contacts between the A3 promoter and the polymerase with DMS and ethylnitrosourea according to techniques developed by Gilbert *et al.* (ref. 10 and unpublished data). Almost all contacts identified lie upstream from the unwound region (unpublished).

The new technique described here can detect any transiently opened AT base pairs; it should be useful in determining whether certain enzymes unwind DNA and, if so, which segments of the DNA they unwind.

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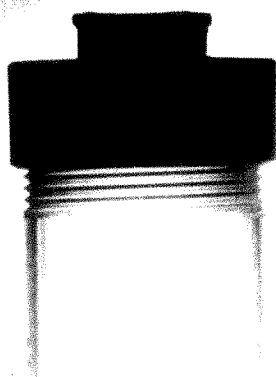
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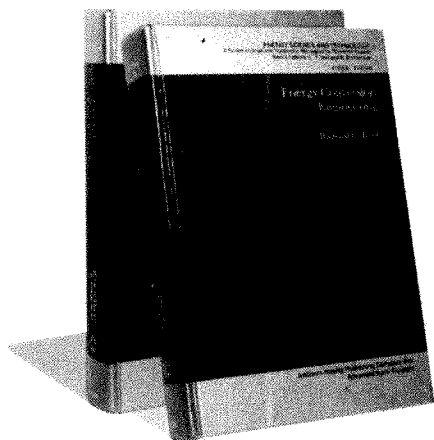
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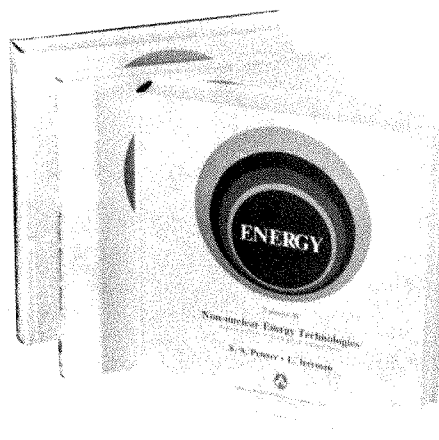
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# reviews

## Analytical history of ornament

N. K. Humphrey

*The Sense of Order: A Study in the Psychology of Decorative Art.* By E. H. Gombrich. Pp. 411. (Phaidon: Oxford, 1979.) £15.

*The Psychology of Decorative Art* is the title of a book which has still to be written. That Gombrich should have used it as the sub-title of his latest essay in *Art History* is a pity for two reasons: first because it will mislead the reader as to what the book is really about; and second, because it has apparently misled the author into biting off more than he can chew. Despite its pretensions this book has very little of value to say about psychology. It has, however, a great deal to say about the more traditional subject matter of the art historian: craftsmanship, style, symbolism, tradition, patronage, and especially about what other critics have had to say about these things.

The body of the book is an analytical history of ornament. From time immemorial *homo faber*—man the serious-minded maker of useful artefacts—has openly cohabited with a more flippant mistress: where he might otherwise have been content with objects which were merely functional, she, his playful (and sometimes tiresome) muse, has persuaded him to decorate them. Her nature, it seems, abhors a visual vacuum; wherever she sees an empty surface, a hard edge, a simple corner, she yearns to overlay it or surround it with decorative patterns. So floors and walls are covered with geometrical designs; an arabesque runs around the cooking pot; the hilt of a sword is decked with jewels; stone columns are fluted and topped with a wreath of acanthus leaves; even the letters of the alphabet are adorned with knots and flourishes.

Gombrich brings to bear on these and related examples of human eccentricity his unrivalled combination of erudition, curiosity and taste. The first chapters deal with the criticism of ornament, the cult of purity and the debates about design in Victorian England. Next the craftsman's practice of pattern making is discussed as a response to the challenges of material, of geometrical laws and visual constraints. And, in perhaps the most intriguing sections of the book, the origins and fate of certain traditional motifs are followed through (the Paisley pattern,

the acanthus scroll, and others). An Epilogue reviews some of the analogies between the spatial orders of decorative design and the temporal patterns of the dance, of poetry and, notably, of music.

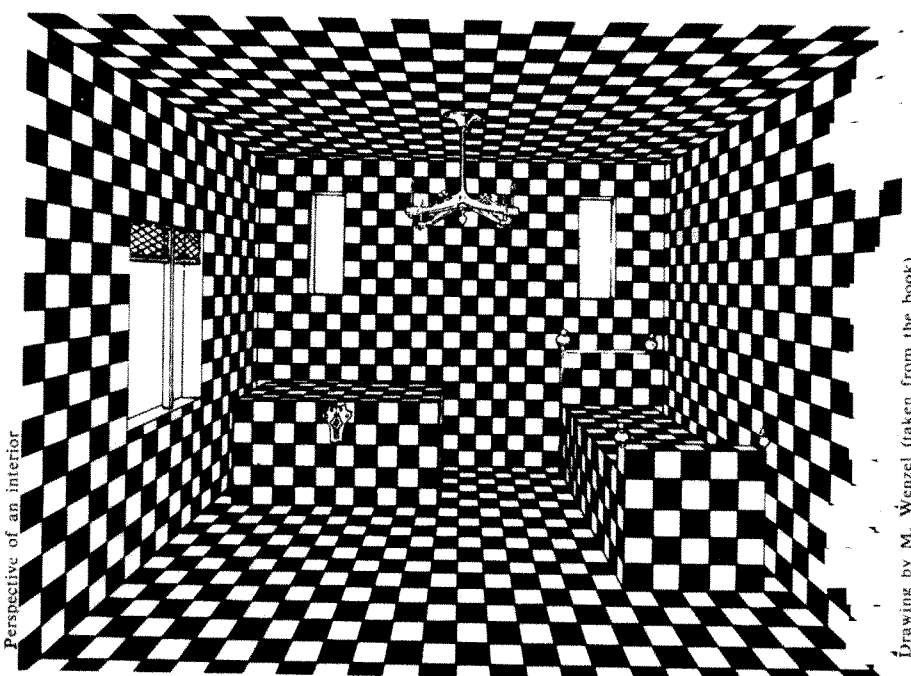
Amidst all this there is, let it be said, a sprinkling of psychology, or what might less kindly be called 'psychologising'. Reference is made (particularly in three middle chapters) to 'gestalt formation', 'selective attention', 'information theory', and so forth. But—and I do not think it is merely the closed-shop mentality of a professional psychologist which makes me say it—I found these excursions into experimental psychology for the most part trite, irrelevant or amateur. Although no-one has yet made a good job of linking art and psychology, Gombrich himself in his earlier book, *Art and Illusion*, has already made a much better job of it than he does here.

To the reader looking for a psychological explanation of design, however, the biggest disappointment will come with the vacuity of the 'biological' arguments which Gombrich propounds at the beginning of the book. It may be true that man and other animals have an innate 'sense of order', and that they find order relaxing and a mild

degree of disorder interesting (*ergo* pleasurable?). But Gombrich does not present a convincing case for why this should be so; nor, having set out the thesis, does he draw any but the most banal conclusion from it—that people like what we already know they like. For me at least, it adds nothing to the understanding of man's love of order to point out that "Nature around us is throbbing with complex rhythms, and these rhythms serve the purpose of life". True, man's heartbeat is rhythmical, and he would be dead if it were not; but, equally, his blood is red and he would be dead if it were not—and that surely does not explain why he prefers the colour red to blue.

The book is finely illustrated, and, as always, Gombrich proves a brilliant guide as to what to see and why. I shall never look at the roof of King's Chapel, or a William Morris wallpaper or a Paisley pattern tie in quite the same way again. But the way in which Gombrich succeeds in deepening our perception of these patterns is by telling us more about their makers rather than by telling us more about ourselves. □

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Drawing by M. Wenzel (taken from the book)

# Disaster strikes from the blue

Peter J. Smith

*Earthshock.* By Basil Booth and Frank Fitch. Pp. 257. (Dent: London, Toronto and Melbourne; Walker: New York, 1979.) Hardback £6.95, \$11.95; paperback available from Sphere (London) in May, 1980.

WITHIN ten minutes of taking up this book I had come to two conclusions, both minor in themselves but both, it subsequently became clear, indicative of deeper malaise. The first was that someone has seen fit to impose a veneer of silly sensationalism on an already spectacular topic, presumably in the belief that a bit of spurious exaggeration does little harm to sales. It begins with the title. An earthshock, it seems, is simply a natural disaster. A short snappy title is all very well, and even to be applauded, but not at the expense of accuracy. As not all natural disasters (for example, glaciation) can be remotely regarded as "shocks", the authors are reduced to referring to "slow earthshocks", which reminds me of that superb character of childhood rhyme, the "bare-footed man with clogs on [who] came slowly running past".

Then there is the lurid dust jacket, showing a huge "tidal wave" about to overwhelm a Manhattan-style environment. Well, all right. More importantly, however, there is the jacket blurb which asks, "can the Earth survive?" and asserts that "A single great natural disaster . . . could destroy civilisation as we know it". For a start, no-one outside Monty Python refers any more to "civilisation as we know it", at least not without inviting ridicule. Yet the basic fault here is not some blurb-writer's exuberance but the authors, who adopt the same tone in parts of the text. They begin the book, for example, with a crude five-page account of the destruction (in 1987) of America's eastern seaboard by a fireball and its aftermath, and subsequently discuss nation-scale disaster from a serious scientific point of view. Is it worth spending time thinking about such unlikely (but admittedly possible) catastrophes when we cannot even cope adequately with events that are more familiar?

The second conclusion is that the book contains far too many flow-stoppers, ranging from the trivial to the more serious. The Earth's crust, mantle and core are referred to throughout somewhat eccentrically as

Crust, Mantle and Core. "Dilatancy" has become "dilateness". "Pangaea" appears as "Pangea"—a perfectly acceptable, indeed desirable, simplification in the context of the abolition of all ligatured diphthongs and their remnants, but presumably a mistake in a book that refers to aeolian, Palaeozoic and palaeomagnetism (and even, in one place, the idiosyncratic "palaeomagnetic"). Imperial units (feet, miles, and so on) are used throughout without even the parenthetical acknowledgement of metric units—a disgrace when (outside North America) a whole generation of children has been brought up on metric units. And even more seriously, one is continually being brought up sharply by the almost impenetrable: "However academic the study of plate movement may seem, its importance cannot be minimised". What does this mean?—the very opposite of what it actually says?

If that were the sole extent of the problem I could perhaps be accused of nit-picking. Unfortunately, however, the confusion goes much deeper, ruining some good ideas in the process. For example, before tackling natural disasters as such, Booth and Fitch devote some 50 or so pages to spelling out the nature of the generally more gradual processes involved in the Earth's behaviour. This is an excellent approach. All too often the authors of "popular" books on natural hazards give the impression that disaster strikes from the blue with little, if any, reference to the context in which terrestrial events and processes can combine to produce catastrophe. But to cover this background in so short a space is difficult and needs careful planning. Booth and Fitch do not entirely succeed. This section is rather confused, dense in concepts and terms all too frequently inadequately explained, and thus likely to be hard going for the non-expert.

The remainder of the book is devoted largely to particular natural hazards—to their general characteristics and to specific examples of disasters they have generated. Here the going is easier. To me, these chapters become more and more engrossing as the material becomes less and less familiar during the progression from earthquakes, through volcanoes, glaciation and flooding to extraterrestrial bombardment. But individual readers will have their own interests. To be objective about it, the book is probably most authoritative on those topics—especially volcanic matters—closest to the authors' hearts. On subjects closer to my heart, on the other hand, respect for the facts is not always all it should be. The notorious Denver earthquakes of the 1960s were not noticed by US

Geological Survey scientists in 1966 but by the alarmed inhabitants of Denver some years before; and the cause of the shocks was discovered by David Evans, a consultant geologist, in 1965. Moreover, the cause was not the injection into the ground of "industrial" waste fluids but of military waste material. The emotional overtones of the affair were thus rather different.

Some of the authors' judgements are also questionable. To say, as Booth and Fitch do, that "If the scientists involved in this work [that is, earthquake studies] are given sufficient encouragement and adequate financial support there is no reason why earthquake prediction should not become a routine matter within a decade" is really far too optimistic and therefore grossly misleading to the uninitiated. Indeed, it seems an unlikely prognosis even in respect of geologically comparatively simple areas such as the San Andreas fault zone of California. Moreover, to give the impression that prediction is purely or even primarily a scientific problem is dangerous. The social implications of a viable earthquake prediction capability are so complex that there must be real doubt whether non-totalitarian régimes could cope adequately with such a power without significant, and perhaps unacceptable, changes in socio-political attitudes.

Failure to give adequate attention to such factors is to demonstrate political naivety, a condition not uncommon among academic scientists. It is also naive to suggest, as Booth and Fitch do, that "the United Nations should have a single, comprehensive world natural disaster control agency with certain overriding powers, able to investigate and prepare for all types of natural disaster anywhere in the world, ready to go in with the right kind of advice and aid immediately it is required, without political let or hindrance". The United Nations has been conspicuously unsuccessful in almost everything it has ever tried to do precisely because such supranational authority is totally unacceptable to any nation of any consequence; and perhaps there is some merit in that uncooperative attitude when one takes account of the motives of the supranationalists. As Booth and Fitch put it, "people must be prevented from falling victim to their own or other people's stupidity and avarice by strictly enforced laws". Nasty.

Despite its descriptive merits, the long-awaited *Earthshock* is at best a disappointment and at worst a bit of a disaster. □

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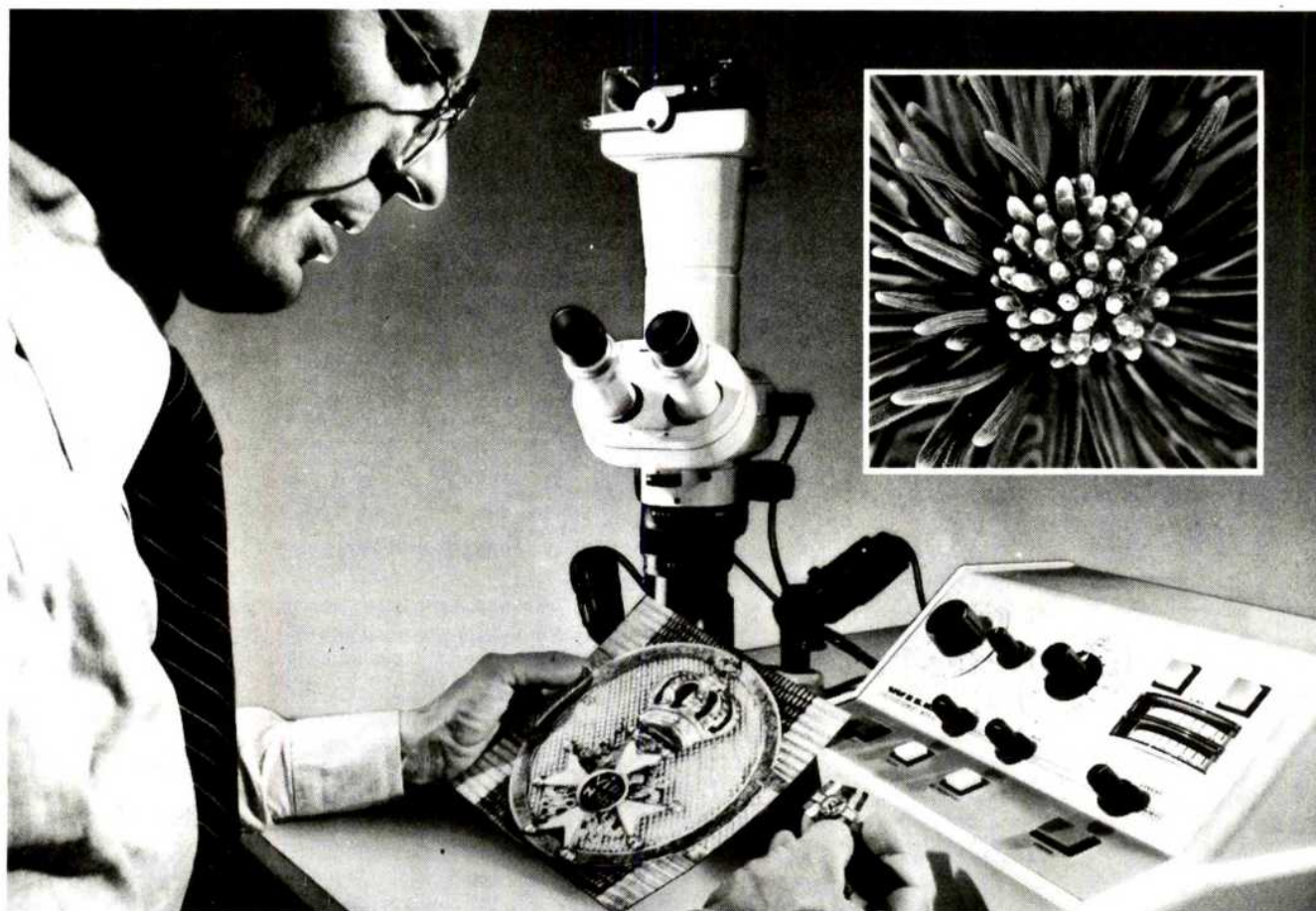
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# Travelling through time

Stephen Siklos

*Time Warps*. By John Gribbin. Pp. 167. (Dent: London, Melbourne and Toronto; Delacorte/Eleanor Friede: New York, 1979.) Hardback £5.95, \$8.95; paperback available from Sphere (London) in September, 1980.

*Time Warps*, the most recent addition to Dr Gribbin's list of popular science books, is about time and time-travel. It is broad in its scope; it begins with a discussion of time-telling through the ages, ends with an excursion into Taoist philosophy and the *I Ching*, and touches on Special Relativity, black holes, precognitive dreams and reincarnation in between.

For the most part, the author does not claim to offer any new insights; rather, as he says, to present old ones in a logically ordered structure. These ideas have been collected either from other popular science books, or from science fiction literature, and the book is liberally sprinkled with direct quotations from these sources. The author's own contribution consists of an explanation of déjà-vu experiences, all forms of dreams, and reincarnation in terms of travel "sideways in time". He postulates the simultaneous existence of all possible universes, and his theory is that dreams and similar phenomena arise from psychic contact with occurrences in some other universe, perhaps one very similar to our own. The beauty of this is that it explains not only dreams which come true, but also those which don't. Dr Gribbin may or may not be the first person to have thought of this idea, but as far as I know, he is the first to admit to it.

This book is aimed at a wide non-scientific audience. It is therefore particularly reprehensible that some of the most important scientific statements are wrong. For example, most of section II is based explicitly on the following incorrect argument. The steps are: (1) Einstein's Theory of General Relativity predicts that under some circumstances massive objects such as stars will collapse to form rotating black holes; (2) these black holes are described by the Kerr metric; and (3) one can therefore travel through a rotating black hole into a new universe. Statements (2) and (3) are false. The misunderstanding arises because the Kerr solution contains paths connecting the interior of a black hole with other regions of the solution, which could be

construed as new universes. Unfortunately, this solution is both vacuum (that is, it contains no matter) and stationary, so it does not represent a realistic collapse. Nor can it have a real observer moving in it (let alone a spaceship). Furthermore, perturbation analysis shows that solutions which resemble Kerr outside the horizon, but which could represent a realistic collapse and contain observers, cannot be extended through the inner horizon into a new universe. The point is that even infinitesimal deviations from the special symmetries of the Kerr solution (such as would be caused by the existence of a particle of matter) drastically alter the structure of the interior solution and destroy the supposed gateway to the new universe (see McNamara, *Proc. R. Soc.* **358**, 499; 1978). It is therefore generally believed that a rotating black hole singularity is not as Gribbin puts it "a different kettle of fish" from a non-rotating one—it is the same kettle. Travellers falling through the horizon could never escape and would eventually be crushed. Appropriately, the incorrect claims made in the book are accompanied by the wrong diagram: the Penrose diagram of a non-rotating charged black hole is shown.

The remaining passages about physics are, on the whole, accurate. These include good introductions to elementary quantum effects, and to the time dilation effects of Special and General Relativity, although even these are marred by a grotesque analogy between velocities and angles on a circle ( $v < c; \theta < 360$ ), and also by the statement that time for an astronaut in closed orbit goes slower than time on Earth: it goes faster if the orbital radius is greater than one-and-a-half times the Earth's radius.

The passages not concerned with physics are characterised by their superficial and tendentious style. The description of Stonehenge as an astronomical computer, which opens section I, is typical. It is gleaned largely from the books by Hawkins (*Stonehenge Decoded*; Souvenir: London, 1967) and by Hoyle (*From Stonehenge to Modern Cosmology*; Freeman: San Francisco, 1972). No mention is made of the review by Professor R. J. C. Atkinson, entitled *Moonshine on Stonehenge* in which much of Hawkins' book is shown to be scientifically and archeologically unsound (*Antiquity*, 1966) or of the correspondence in the 1967 volume of *Antiquity*, where comments range from "dubious" to "untenable". The section then progresses to the subject of clocks, which is illustrated by a picture of a sundial, apparently capable of "all the accuracy

of a modern clock". It concludes with a discussion of time paradoxes and the meaning of time. The level of debate here, as typified by the subsection headed "Temporal Pigeon Holes and the Cosmic Postman", is most politely described as lightweight.

Clichés, polysyllabic humour, and other forms of turgid jocularity abound in this section. If you get past "Meanwhile, the poor man in the street was harried along, willy-nilly, into the 20th century", you are confronted with "intrepid circumterrestrial travellers", their "staid counterparts back home", the "change of date situation" and the fact that "even the common man and woman must come to grips with the subtleties of horological hairsplitting".

More serious, however, is the pernicious tendency to populate the book with 'goodies' (in whose work the author sees support for his theories) and 'baddies'. Thus the goodies are "respectable physicists, schooled in the best scientific tradition", "very solid members of the community", and "very sober scientists with impeccable academic credentials and years of research experience". On the other hand, we read that "Many theorists have an innate dislike for white holes, perhaps because they are only just coming to terms with the implications of black holes, and don't want to move too fast too soon"; that "archeologists cannot bring themselves to accept the subtleties which astronomers find in the Stonehenge computer"; that "there is not enough evidence yet to persuade the doubters" (for the existence of tachyons); and, incredibly, that Dr Gribbin sometimes wonders whether the "verbiage" in philosophical articles is not just "window dressing, to frighten off . . . people who are not 'philosophers'". Interested readers will find plenty more similar examples throughout the book.

Much the same can be said of section III, which purports to relate Eastern Philosophy to Western Science. To be fair, I think that this section will appeal to people with a taste for bizarre interpretations, and many readers will want to follow up the ideas in the bibliography provided. I cannot take these interpretations seriously, because they fail to explain the basic mechanisms and so seem to have no advantage over many less outlandish theories.

A glance at this review will reveal that *Timewarps* is likely to become a best-seller, and that many people will learn a lot of new ideas from it. It is a pity that it was not written with greater regard for accuracy. □

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# Sophisticated astronomy

Euan W. MacKie

*Megaliths and Masterminds.* By P. Lancaster Brown. Pp. 246. (Robert Hale: London; Scribners: New York 1979.) £5.50; \$14.95.

THE title suggests that here is another attempt to capitalise on the increasing interest, at many levels, in the evidence for sophisticated astronomical practices in the monuments of Neolithic and Early Bronze Age Europe. There must be at least a dozen books on the market at present with similar titles, and each with a picture of Stonehenge or some other megalithic site on the jacket, so the reviewer may be forgiven for having become somewhat hard to please in this area. These books vary considerably in quality, from the frankly mystical, through orthodox archaeological textbooks to excellent explanations of a difficult and complex subject (see *Nature*, 275, 75; 1978). One may well ask, what is the purpose of another one unless it includes original research or new ideas?

Mr Lancaster Brown in fact ranges more widely than the megaliths of the title, which he correctly defines as including both the standing stone sites of Britain and Brittany and the chambered burial mounds of Atlantic Europe from Spain to Scandinavia (although the latter also include dry-walled constructions and, if the collective burial rite is thought to be the decisive linking feature, some rock-cut tombs as well, which are in no sense megalithic). He has chapters on almost everything connected with astronomy in the ancient world, starting with Alexander Marshack's 'lunar notation' scratches on Palaeolithic bone implements. However, his suggestion that these marks, dating to well before 12,000 years ago, may be ancestral to the Irish Ogham script of less than 2,000 years ago will scarcely find favour with philologists and simply shows that speculating in the historical disciplines can seem too easy to the layman.

The author is, however, very interesting on the early development of research into archaeoastronomy and

ancient metrology, and this is perhaps the most useful part of the book. We are given vivid accounts of Sir Norman Lockyer, L. Piazzi Smyth and Sir Flinders Petrie at work among the temples, pyramids and obelisks of ancient Egypt and of the evolution of thought about astronomical qualities in British prehistoric sites from the seventeenth century onwards. It is very useful to be reminded that many of the modern ideas about ancient astronomy and metrology, developed in such detail by the Thomms, in fact derive from those of earlier workers, and salutary to learn again that some of these became obsessed with their theories to such an extent that they ignored or derided the historical and archaeological evidence. It is true that archaeologists were often of little help, usually being innately sceptical about archaeoastronomical ideas, but they were sometimes faced with such amazing nonsense that their scepticism is understandable.

The author provides one classic example from Lockyer's 1909 book on Stonehenge in which he discussed the living quarters of the astronomer-priests who, he assumed, were at work in Neolithic Britain. He supposed that damp was a severe problem so that shelters were needed, but assumed quite arbitrarily that the people of 4,000 years ago were entirely ignorant of carpentry. Thus they had to live in the megalithic chambered tombs of the period which were supposed to have been equipped with stone doors. (One would scarcely be surprised to read further that the doors often jammed, thus explaining the skeletons frequently found inside!) Even 70 years ago British archaeology had advanced far beyond that; presumably Lockyer had never entered a museum and seen a stone axe. Memories of this kind of thing still help to perpetuate a divide between workers like the Thomms and many orthodox archaeologists.

These historical insights are very useful but the chapters on the astronomy of the ancient Babylonians and of the American Indian civilisations are too brief to be of more than casual interest. Presumably the section on ley lines and 'geomancy' was included as another warning about the eccentric ideas that still flourish on the fringes of megalithic astronomy and which also help to keep many archaeologists at a distance. Perhaps this very breadth of coverage conceals another danger. The author often gives guidance and opinions on the present state of our knowledge of Neolithic Western Europe but these are based primarily on the technical archaeoastronomical evidence, itself only a small fragment of the total knowledge we have of the period concerned.

For example, there is not one but two vitally important aspects of hypotheses about the 'masterminds' among the megalith builders. The first of course concerns the plausibility of the sites claimed as observatories, or as showing high meteorological skill in measuring, and has been discussed extensively many times: this book deals mainly with it. However, the question of what kind of social organisation the people concerned had is an equally important but much trickier subject; it is rarely touched on by archaeoastronomers and their followers as it depends mainly on more orthodox archaeological evidence as well as on anthropological knowledge. There is no problem in ancient Egypt and Babylonia, or in Mesoamerica, which were urban societies known to have had specialist classes of priests and wise men, but what about Neolithic Europe? The population there was in a pre-urban stage and the usual view is that it was much more simply organised, not stratified into classes, and that it certainly did not possess élite orders of astronomer-priests and wise men of the kind which should have existed even if only half of what Professor Thom claims is correct.

The problem is whether the most important Neolithic sites and artefacts can be reinterpreted in terms of such a stratified, theocratic society. If none can, then the case for sophisticated ancient astronomy becomes much harder to maintain, no matter how impressive the technical evidence. The reviewer recently devoted half of an entire book (*Science and Society in Prehistoric Britain*, 1977) to raising this problem, and to making a start on resolving it, but feels he still has some way to go before convincing all his colleagues. Mr Lancaster Brown, however, only brings up the problem in a few lines in the final chapter, saying simply that we can be sure that such an élite class existed and that "Archaeological field evidence strongly supports this idea". Neither the book cited, nor Aubrey Burl's fundamental work *The Stone Circles of the British Isles* (1976) is mentioned anywhere and one must regretfully conclude that this is a further example of the idea that it is enough to be familiar mainly with the specialised archaeoastronomical evidence. Yet that is only the start of the journey, providing a new and clear window on to the past in addition to many older ones, by means of which our very complex but fragmentary picture of life in Neolithic Britain and Europe may eventually be substantially modified. □

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● In the review of *Physical Chemistry: Principles and Applications for the Biological Sciences* by Tinoco, Sauer and Wang, (*Nature*, 278, 85; 1979), it was incorrectly stated that a paperback edition had been published. A hardback edition only is available from Prentice-Hall International at £12.95, with a paperback *Solutions Manual* at £2.55.

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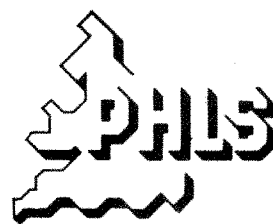
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## Biological regulation processes

*Biological Regulation and Development*. Vol. 1: Gene Expression. Edited by R. F. Goldberger. Pp. 558. (Plenum: New York and London, 1979.) £24.88.

THE motivation to produce this inaugural volume of a multi-author treatise on regulation is that it would be productive to approach the subject in a way that cuts across traditional boundaries. The editors aim to avoid bringing together all possible facts relevant to a particular operon, virus or biosynthetic system. For instance, no one person works on suppression *per se*, only on aspects of the phenomenon. The actual chapter by Steege and Söll on suppression successfully picks up the various threads from many different laboratories. This approach, however, means that the same system is often discussed from different points of view by different authors. In a few places this needs some stronger editorial control and cross reference to assist readers with less experience in these areas. Dia-

grams of the same system sometimes appear quite differently in consecutive chapters. The chapter on regulation of DNA replication by Lark suffers from a complete lack of diagrams. In other chapters there is some obvious duplication of material which can be irritating. On balance, however, these problems do not greatly detract from what turns out to be a stimulating set of contributions stressing the essential concepts that underline our knowledge of regulation of gene expression. The book will be of considerable value to senior undergraduates, to postgraduates and to their teachers. Individual chapters deal mainly with regulation in microorganisms but recurrent themes are evolution of regulatory mechanisms and to some extent the relationships between prokaryotes and higher organisms. Later volumes must expand on this latter aspect but perversely no indication is provided, at least in this volume, of future contents.

The initial chapter by Goldberger covers general strategies of genetic regulation and provides a frame of reference for the discussions of operon complexity by Campbell and autogenous regulation by Savageau. Clarke develops the evolution theme with the

view that regulatory systems can be potent agents of evolutionary change. The chapters that follow deal with the relevance of DNA sequence and conformation. Pribnow's chapter on control signals should really be followed by the two more physicochemical contributions from Von Hippel and Sobell on the specificity of DNA-protein interactions and implications of DNA conformation. These latter chapters, careful consideration. Steitz then though speculative in places, deserve stresses the importance of RNA-RNA interactions, especially in ribosome function and proposes that mRNA effectively selects protein molecules that facilitate such interactions. Cortese follows with a review of the various functions of tRNA that are secondary to, or derived from, its primary function as amino acid adaptor. Finally, Maaløe amply demonstrates the stimulating but complex challenge of evaluating the possible interplay of all these various regulatory mechanisms in the growing organism (mainly *E. coli*).

Roy H. Burdon

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## Thermal neutron scattering

*Introduction to the Theory of Thermal Neutron Scattering*. By G. L. Squires. Pp. 260. (Cambridge University Press: Cambridge, London, New York and Melbourne, 1978.) £16.

THERMAL neutrons are used widely to study structures and correlations in solids and liquids. They have just the right wavelength for atomic physics; and, as nuclear scattering takes place effectively at a point, one can use the scattered intensity to disentangle the motion of atoms. The momentum transfer dependence of the scattering is the Fourier transform of the spatial structure, and the energy dependence is likewise the Fourier transform of the time dependence of correlations. The magnetic moment of the neutron is a valuable bonus, sensitive to the magnetic structure of materials at the atomic level.

There are several textbooks on the physics which emerges, and on the techniques, but most authors content themselves with rough and ready derivations (or simply quoting) the rather complicated expression for scattering cross sections which are needed for quantitative analysis. This can be hard on research students wanting to extrapolate formulae to fresh situations and

wanting to grasp from first principles the meaning of phonons, magnons, spin waves and correlation functions. Gordon Squires' book fills this gap admirably. As the title implies, the book is aimed at deriving the formulae systematically and rigorously. It is the Goldberger and Watson of thermal neutron physics. Students will appreciate the beautifully precise, lucid and explicit algebra, which follows smoothly from undergraduate quantum mechanics. The material is extraordinarily well organised; there is great care over nomenclature, no mistakes and no missing lines. On the other hand, the casual reader might find the subject a bore, and even the dedicated reader will have to reflect carefully on the objectives of the formulae and the physical implications of each factor or approximation. To help his understanding, there are several problems, many of them quite hard, with solutions. Formulae are illustrated briefly with experimental results, but there is no systematic attempt to explore the physics which emerges from thermal neutron scattering.

This volume will be a valuable prop to the experimenter wanting to be sure of the foundation of his subject.

D. V. Bugg

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## Microbial life in extreme environments

*Thermophilic Microorganisms and Life at High Temperatures.* By T. D. Brock. Pp. 465. (Springer: New York, Heidelberg and Berlin, 1978.) \$29.70; DM54.

THIS book collates the ecological studies Professor Brock initiated and directed on microbial life in the hot springs of Yellowstone, some 1,200 miles from his University campus and at an altitude where water boils at 92 °C. The scope of the project was admittedly limited initially, but in the light of discoveries of unknown microbial species adapted to live in a variety of extreme environments, research ramified to include man-made thermal habitats and hot springs on a worldwide scale.

The objective was to study the structure, biochemical and growth characteristics, evolution and dispersal of such bacteria, blue-green algae and eukaryotic algae which seem to set the limits for life with respect to pH and temperature. Such natural model systems, with the relative constancy of their features, would then make possible deductions about long term ecological consequences of man-produced thermal and chemical pollution of the environment.

The cover picture showing the run-off channel of a Yellowstone hot spring is more suggestive of the scope of the book than its title. *Exploring Microbial Life in Hot Springs* might be a more appropriate title, which better expresses the 'adventure' character of the book.

The book stamps a record of personal achievement by drawing a demarcation line from earlier or contemporary work; it aims at helping future researchers, especially in Yellowstone, with the detailed description of the sites studied (chemical, physical and topographical). Furthermore, it includes valuable unpublished observations, suggests areas for future research and provides a wealth of ideas for *in situ* research approaches and improvised techniques that proved useful where conventional methods could not work or were unavailable. The author's confessions of being often misdirected or "fooled", for example, by *Thermoplasma*, when in fact a new bacterium, *Sulfolobus*, had been isolated, illustrates the complexity and very often deceptive nature of working in an area where no precedent existed.

The seminar style of the book gives the reader both pleasure and a good share of the fascination and excitement

of the field work. The personalised manner in which the book was written is highlighted by the author's comments on his original paper on the genus *Sulfolobus*, that was rejected twice by the *Journal of Bacteriology*; and by the final chapter "Some Personal History" that includes a family and research team photograph.

The book illustrates effectively how research orientation can become usefully multi-directional in the way discoveries developed in a multi-disciplinary fashion, thereby becoming of interest to ecologists, environmental scientists, biochemists and geologists

alike. The author did not even miss the opportunity to comment on his observations on the effects of pH limits on the existence of higher organisms, including insects, frogs, birds, fish and plants.

Finally, this book, if read from cover to cover rather than used as a reference source, has all the elements that make it useful to ecologists. It would also make inspiring introductory reading for new researchers in microbial ecology.

**G. D. Anagnostopoulos**

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## Liquid structure in relation to their crystalline solids

*The Molten State of Matter: Melting and Crystal Structure.* By A. R. Ubbelohde. Pp. 454. (Wiley: Chichester, UK, and New York, 1979.) £23.50.

THIS book may mark the end of an era: or it may not. Scientific eras overlap, and a severe decimation of the time scale may be needed before the transition between them is marked with any definiteness. Knowledge of the figure of the Earth did not much assist the search for the sources of the Nile, and the Nile is still imperceptible on the geoid. This book is about liquids, and Ubbelohde has chosen its title to emphasise the fact that he will consider their structure in relation to that of their crystalline solids. Thus, attention is focused on phase change, and the relationship between the phases between which the change occurs: but one will find no mention here of the renormalisation group, or the names of Peierls, Landau, Wilson or Kadanoff, nor even, rather surprisingly, of Bernal and Fowler: which is not to say that there is not a wealth of references here to work in the present decade as well as past decades. Conversely, one may read a book about the renormalisation group and find mention of only three or four particular substances, if that. Ubbelohde deals with particular substances, and many comparative tables of the melting parameters of particular classes of substance are a feature of the book. These are instructive, and would be more so if one knew how much confidence to place in them. They seldom contain estimates of error, and sometimes fail to indicate source: there are discrepancies, sometimes minor and sometimes substantial, between values for

the same physical quantity in different tables: for example, the entropy of fusion of NaCl is 6.7 e.u. in Table 8.1 and 6.23 e.u. in Table 8.34. There are quite alarming discrepancies between heat capacity data for solid and liquid alkali metals in Table 9.1 and data for sodium in Fig. 9.1. Contradictory information about the magnetic susceptibility of iron phases at high temperature is given in Fig. 9.7 (p263) and the text on p264.

On p103 the reader is told, as though he should believe it, that for a second-order phase change two free energy surfaces must touch and intersect, with never a mention of bifurcation. Naïve readers must be warned against chapter 14, on "Liquid Crystals", shot through with misconceptions which should never have survived the classic paper of G. Friedel (1922).

Pleas for more observational data are a recurring refrain, and often justified: but, although on p90 the author complains about the lack of information regarding domain formation in order-disorder transformation, he never mentions antiphase boundaries, a keyphrase which could have led him to a wealth of material in the metallurgical literature.

It becomes apparent that several distinct and separate cultures grow on the one substrate of phase transitions. Of these, this book provides a useful literature guide to a substantial part of the physicochemical culture. Those readers who will take the trouble to check and amend the data will be in possession of a well-planned survey of empirical melting parameters, and some liquid properties for various classes of material. Considering the price to be charged, this should have been attended to by the publisher's editor.

**F. C. Frank**

*Sir Charles Frank is Emeritus Professor of Physics at the University of Bristol, UK.*

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MILBURN, John A. Water Flow in Plants. (Integrated Themes in Biology.) Pp.xi+225. ISBN-0-582-44387-3. (London and New York: Longman, 1979.) £6.75 net.

MONOTREME BIOLOGY. (Proceedings of a Symposium held in Sydney in May 1978.) Pp.257. (Mosman, N.S.W.: The Royal Zoological Society of N.S.W., Taronga Zoo, 1979.) \$A5 plus postage.

NICOLINI, Claudio A. (ed.). Chromatin Structure and Function: Molecular and Cellular Biophysical Methods. (NATO Advanced Study Institutes Series, Series A: Life Sciences, Vol. 21, Part A.) Pp.xix+1-368. ISBN-0-306-40075-8 (Part A). (New York and London: Plenum Press, 1979. Published in co-operation with NATO Scientific Affairs Division.) £20.47.

NICOLINI, Claudio A. (ed.). Chromatin Structure and Function: Levels of Organization and Cell Function. (NATO Advanced Study Institutes Series, Series A: Life Sciences, Vol. 21, Part B.) Pp.xix+369-880. ISBN-0-306-40076-6 (Part B). (New York and London: Plenum Press, 1979. Published in co-operation with NATO Scientific Affairs Division.) £26.77.

PARK, Joon Bu. Biomaterials: An Introduction. Pp.x+251. ISBN-0-306-40103-7. (New York and London: Plenum, 1979.) £22.50.



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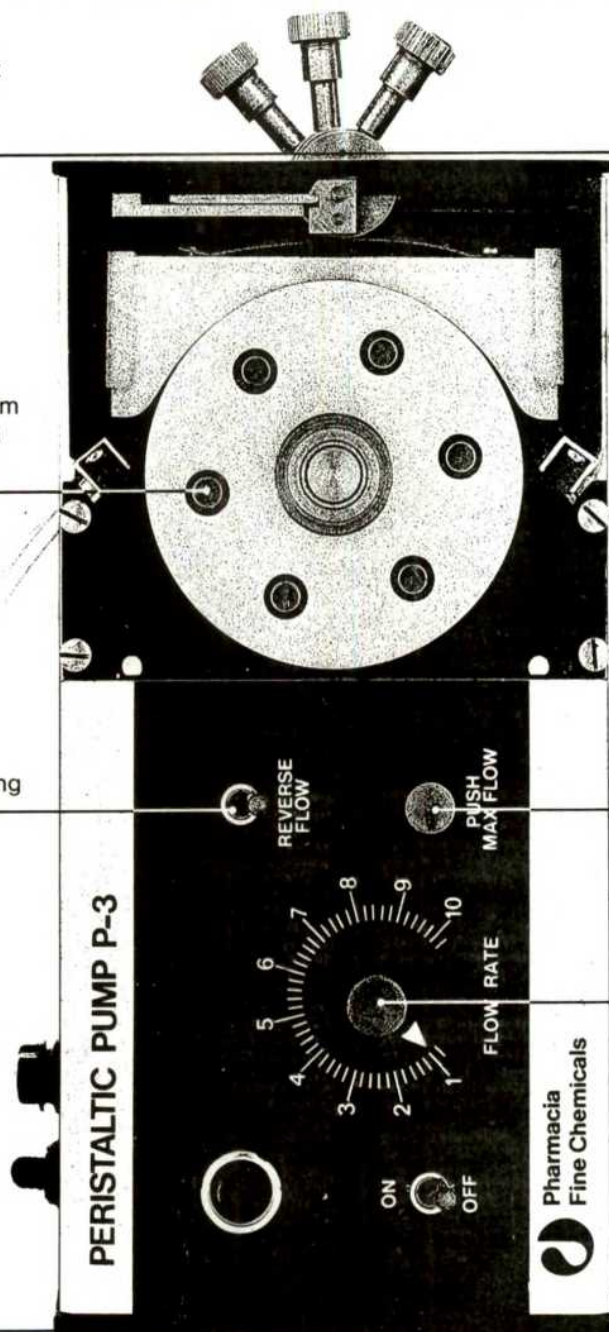
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# obituary

## D. A. Hems

TO BE a successful scientist requires three attributes: intelligence, hard-work and perspicacity. Intelligence and hard work are not enough; perspicacity, insight, flair—call it what you will—make up a somewhat undefinable third requisite. Dr D. A. Hems, Reader in Biochemistry at St George's Hospital Medical School, London, who died tragically on 2 February 1979, possessed all three qualities. He was just 40, at the peak of his scientific career.

Hems received his schooling at the John Lyon school in Harrow, and entered University College, London as Bucknill Open Scholar in Medicine. At the end of his pre-clinical years, he took an intercalated Hons BSc in Physiology; during his clinical training at UCH he received prizes in neurology, and in obstetrics and gynaecology. On qualifying, he decided to pursue a scientific career, and moved to the Institute of Psychiatry to work on biochemical aspects of brain metabolism under Dr (now Prof.) R. Rodnight; three years later he received his PhD. Intelligence and hard work were clearly two of Hems' attributes.

Hems' interest now lay firmly in metabolism. But not in the elucidation of intermediates or in the properties of isolated enzymes. He realised that this aspect of biochemistry was, by 1966, proceeding on fairly predictable lines. What was lacking, was a proper correlation between the physiological behaviour of an organ and the biochemical activity of its cells. In Oxford, Sir Hans Krebs was developing the technique of whole organ perfusion. So it was to Oxford, on an MRC Research Fellowship, that Hems went in 1966: the first sign of that third attribute.

In 1969 Hems was appointed Lecturer in Biochemistry at Imperial College, under Professor Sir Ernst Chain. He now began to develop what proved to be his major scientific interest: the hormonal control of metabolism, using intact organs and cells. It was clear to him, as to others at this time, that the concept of a specific target organ for a specific hormone was too restrictive. He showed, for example, that the antidiuretic hormone vasopressin also has a direct action on the liver; that action is to stimulate glycogen phosphorylase, but not, as Hems discovered, by way of

cyclic AMP. Another of Hems' interests lay in obesity. With characteristic insight he chose to investigate fat synthesis in liver, rather than in adipose tissue: a choice that is beginning to appear well-founded. More signs of that essential third quality; sadly, fulfillment of Hems' potential was not to be realised.

During his time at Imperial College, Hems established close links with the research being carried out by Dr Anne Beloff-Chain (Lady Chain). He also built up an active group of his own; when in 1976 he was offered a Senior Lectureship in the new Biochemistry Department at St George's, the entire group chose to move with him to Tooting. Hems—Doug to all who were close to him—was at once immensely kind and considerate, helpful and inspiring, and it is easy to see why he was able to attract bright and energetic students, as well as more senior research workers. As a teacher, also, Hems was dedicated to his students.

His scientific achievements led to his appointment as an editor, first for *Clinical Science and Molecular Medicine*, and next of the *Biochemical Journal*. He also played an active part in the Regulation in Metabolism Group of the Biochemical Society.

Hems loved music and poetry; he particularly enjoyed witty and pithy comments on life, and was himself gifted with a dry sense of humour. The latter contributed to his demand as a guest speaker: at the time of his death, he had invitations to lecture at Guildford, London, Cairo, Cambridge and Copenhagen. But however much his scientific colleagues will miss him, it is his widow, Phyl Hems, and his two children Jacky and Clare, who face a sad and lonely future without Doug. It is to them that our sympathy is extended.

C. A. Pasternak

## Ralph Emerson

WITH the death on 12 March 1979 of Professor Ralph Emerson, mycology has lost one of its brightest stars. He was born in New York City in 1912. He studied biology at Harvard obtaining his BS in 1933 and his PhD in 1937. He then came to England with a National Research Council fellowship and worked in the botany school at Cambridge for the next two years. In 1940 he joined the faculty of the University of Cali-

fornia at Berkeley becoming a full professor in 1953 and being chairman of the botany department from 1967 to 1971.

He was elected to the National Academy of Sciences in 1970. He served as president of the Botanical Society of America (1967), as president of the Mycological Society of America (1953) and as vice-president of the British Mycological Society (1971). In 1964 he received the Merit Award of the Botanical Society of America. To the First International Mycological Congress at Exeter in 1971 he contributed a brilliant general lecture which ended in a standing ovation.

His greatest scientific contributions were to the biology of water-moulds especially those belonging to the Blastocladales. Hans Kniep (1881–1930), that great student of sexuality in fungi, discovered in the last years of his life a unique sexual cycle in the water-mould *Allomyces*. Ralph Emerson developed the study of sex in species of that genus in beautiful detail. His 'An experimental study of the life-cycles and taxonomy of *Allomyces*' (*Lloydia* 4, 77–144; 1941) has become one of the classic papers of mycology. He was also responsible for a film 'Syngamy and alteration of generations in *Allomyces*', one of the finest mycological films ever produced. Further, Ralph Emerson made important contributions to the physiology of water-moulds. He found that certain species require an environment with a high concentration of carbon dioxide if resting spores are to be formed, whilst in one species oxygen must be absent if any growth is to occur, a most unusual situation in fungi.

He maintained that, although Blastocladales were clearly not organisms of economic importance, their study could make valuable contributions to fundamental problems. He inspired students and colleagues to undertake work that has fully vindicated this view. He launched Dr Cantino on his productive study of *Blastocladia* which has done so much to illuminate the relationship between biochemistry and morphology. He inspired the late Dr Machlis and his colleagues to investigate the hormone concerned with the attraction of male to female gametes in *Allomyces*. This led in 1968 to the



resolution of the structure of sirenin, the first plant sex hormone to be characterised. All over America there are mycologists of distinction who received their early inspiration from Ralph Emerson.

During the war years 1944–46 he served as microbiologist to the Emergency Rubber Project in the United States which led him temporarily into the field of thermophilic organisms, particularly fungi responsible for development of high temperatures in compost heaps. After the war, although Blastocladias remained his main research area, he continued his war-time interest and with Dr Cooney was responsible for an important book *Thermophilic Fungi* published in 1964.

Ralph Emerson was much concerned with the basic causes of the student troubles on the Berkeley campus in the late sixties. In particular he felt it his duty to respond to his students' demands for relevance in mycological teaching. He immediately set to work to organise what he described as 'a running story of fungi and mankind with whatever minimum mycological technicalities had to be provided'. He devised a course that was both academically sound and excitingly relevant.

Ralph Emerson was a man of strong

character and great personal charm. Not only was he a research worker of outstanding ability, but he also wrote and spoke with elegance and clarity. His enthusiasm for his subject and for the experimental approach was unbounded. His former students and mycologists throughout the world mourn the loss of an eminent scientist. Above all he was a very special kind of person.

C. T. Ingold

## David Zimmerman

IT WAS with extreme regret that I heard of the sudden death of Dr David Zimmerman, who died on 10 November 1978 of haemorrhage following an injury to his eye by a tennis ball.

He came to England in the sixties with an MSc in physics from the University of Wisconsin and joined Dr Aitken in the Research Laboratory for Archaeology in the University of Oxford. Here he became one of the most productive research workers in the new field of thermoluminescent dating.

One of the factors which made absolute dating difficult was lack of homogeneity in the samples of ancient pottery to which it could best be applied. Dr Zimmerman developed a way of avoiding this problem by the separating out of grains in the sample which

were small compared with the range of alpha particles and in which therefore a statistical uniformity could be expected.

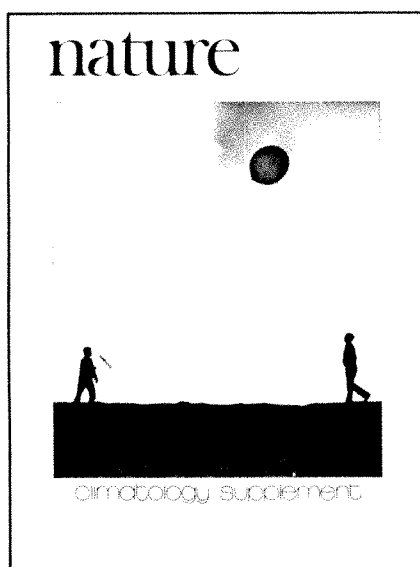
After gaining a well-deserved D.Phil as a result of this work, he returned to the United States where he continued to work on thermoluminescent dating of pottery at Washington University, St. Louis. Here he explored the possibility of using the zircon inclusions which usually contain so much uranium and thorium as to be almost entirely unaffected by background radiations, which are often impossible to estimate accurately.

Dr Zimmerman had many interests outside physics which he applied with characteristic success; for example he obtained a Blue at Oxford for badminton and played tennis with enthusiasm (Intercollegiate-Caltech); it was tragic that this should have led to his death. He enjoyed walking and camping and was interested in piano music. He was forty years old at the time of his death and has left a wife, Joan who was a contemporary research worker in 'TL' at Oxford. During the last twelve months he had started a valuable newsletter *Ancient TL*. His death is a very great loss to the field of thermoluminescence as well as to his large number of friends.

J. H. Fremlin

# nature special supplement Climatology

(23 November 1978)



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- MJ Ingram, DJ Underhill & TML Wigley  
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- GJ Shutts & JSA Green  
*Mechanisms and models of climatic change*
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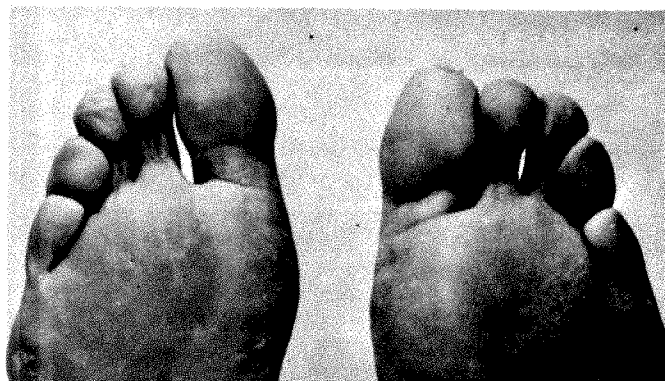
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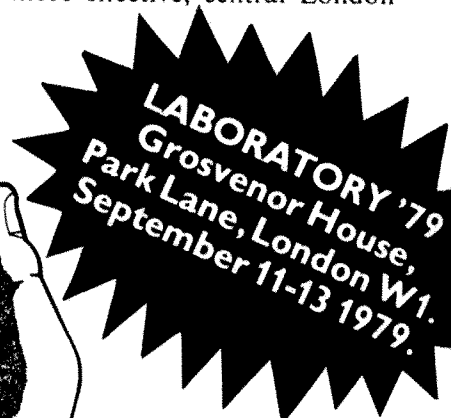
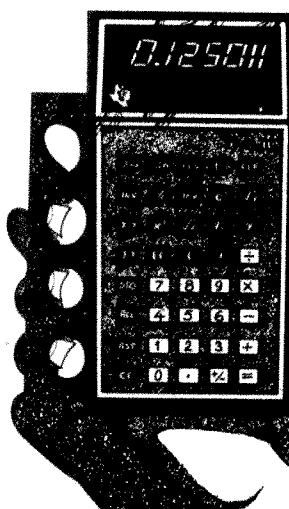
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# ACHEMA '79

ACHEMA 79, the 19th Chemical Engineers' Exhibition-Congress, is to be held in Frankfurt on June 17-23. This AICHEMA news section gives brief notes on some of the products on show. For more details from the manufacturers circle the appropriate numbers on the card bound inside the cover.

## Krupp

At AICHEMA 1979 Krupp Atlas-Elektronik will be presenting the process video system PVS 1050, an improved version of the colour video system PVS 1100 which has been used for some years in the chemical industry. This new system enables a user who already has a process computer to adopt process video technology at relatively low cost. The PVS 1050 does not require a mass storage unit and serves as a terminal for connection to a control computer via standard interfaces. Colour process diagrams, curves, bar charts and texts are displayed on a screen. The diagrams are created interactively with a light pen and keyboard. Chemical pumps require bearing components made of material resistant to chemicals. Mechanical seals made of the new hardmetal grade GTK, a nickel-bonded alloy developed by Krupp Widia, can also be installed in pumps handling corrosive fluids. These are available with diameters of up to 350 mm. They are made of solid hardmetal or fitted with hardmetal rings which are clamped or soldered in position.

Cutting mill knives are used in the plastics industry for on-line size reduction of the constituents of plastics mixes and for cutting up plastics scrap for recycling. These materials have highly abrasive fillers and the knives thus become blunt quickly. Krupp Widia has developed a new highly wear-resistant hardmetal grade and now supplies mechanically clamped cutting elements for these knives. They can be reground several times, ensure economic production, especially with fibre-glass reinforced duro- and thermoplastics, and have service life of up to 40 times that of conventional steel knives.

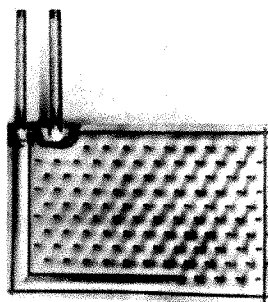
At AICHEMA 1979 Krupp Widia will be exhibiting hardmetal-tipped granulator plates for granulating polyethylene. This innovation increases the service life 15-fold compared with the hardfaced design previously used.

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## Henry Wiggin

The Wiggin exhibit highlights Wiggin's ability to meet all the main international specifications in the manufacture of nickel based alloys for the construction of chemical and petrochemical plant. Among the products to be featured will be the well-known Nickel 200/201, Monel alloys 400 and K500, Inconel alloys 600 and 625 and Incoloy alloys 800H and 825. Recent additions to the range are Corronel alloys B2 and C4 and Alloy G. Alloy G (from Huntington Alloys Inc.) is a high-nickel alloy which, in acidic and alkaline environments, is used for its high level of resistance to pitting and stress-corrosion cracking.

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Senior Platecoil heat exchanger

## Senior Platecoil Ltd

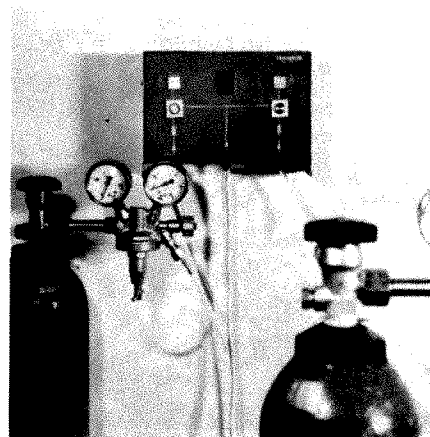
Senior Platecoil Limited of Watford, England exhibit for the first time at AICHEMA 1979 a range of embossed metal plate-type heat exchangers manufactured by their new hydraulic expansion process. Platecoils are used for heating or cooling process liquids by either immersion in process vessels, incorporated into vessel walls as integral sections or clamped externally to vessel walls. Materials of manufacture are normally high grade carbon steel, AISI 316 stainless steel or commercially pure titanium. Other materials of manufacture include, for example, Hastelloy, Incoloy, Monel or other exotic alloys. Heating or cooling media include steam, hot water, hot oil, chilled water or refrigerants. All Platecoils are pressure tested before despatch and operating pressures can be as high as 22 bar g. Platecoils can be shaped, curved, rolled into full cylinders or even provided with cutouts to give the maximum design flexibility demanded by process engineers.

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## Büchi

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Heraeus change-over assembly for gas cylinders

## Heraeus

New from Heraeus is a low-priced assembly for automatic change-over to a reserve gas cylinder. This automatic change-over assembly, type GM, is most useful in those cases where a continuous supply of gas must be maintained with minimum attendance. The assembly is designed for connection to two gas cylinders (or batteries of cylinders), one of which serves as reserve. It changes over automatically to the reserve cylinder as soon as the first cylinder is empty. The fact that the cylinder is empty and should, therefore, be replaced, is indicated by a red lamp. A buzzer can be provided additionally. The signal will stop only after connection of the new cylinder, which will then serve as reserve. Manual change-over is possible at any time. The change-over assembly may be used only in conjunction with compressed or liquefied gases, such as CO<sub>2</sub>, N<sub>2</sub> and O<sub>2</sub>.

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## George Kent Group

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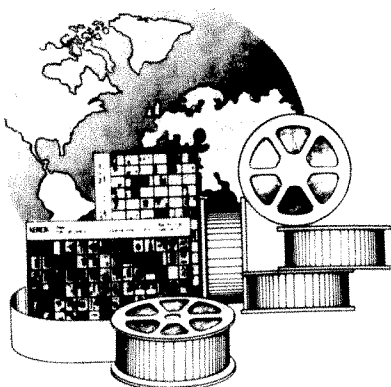
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the BBC Brown Boveri stand. Equipment on display comes from six manufacturing companies in the Kent group—computer based process control systems from Kent Automation Systems, Hitchin; electronic process control instrumentation and flow-measuring equipment from Kent Instruments, Luton; process analytical instrumentation from Electronic Instruments, Chertsey; pneumatic process control instrumentation from Kent-Tieghi, Italy; level instrumentation and electronic recorders, indicators and controllers from Foster Cambridge, St Neots; and gas monitoring equipment from George Kent Electronic Products, Cambridge. On show for the first time is a new generation of integrated electronic process control instrumentation, the P4000 system. Process analytical instrumentation includes equipment for measuring pH, conductivity and dissolved oxygen, and a range of selective ion monitors. Oxygen measuring instruments include the Model 9407 automatic dissolved oxygen system which increases the convenience and reliability of oxygen measurements, even in stagnant conditions, by providing *in situ* remote calibration checks and cleaning. The 8000 series of selective ion monitors is designed for continuous on-stream monitoring of specific-ion concentration. Eight different ions can be identified and measured by models in the series. A new colorimetric analyser for silica will be on show. This is the Model 8061 which provides a continuous readout of silica concentration in the range 0–2,000 p.p.b. This unit has important applications in the power generation industry. A filtration system can be incorporated to allow the monitoring of heavily polluted samples.

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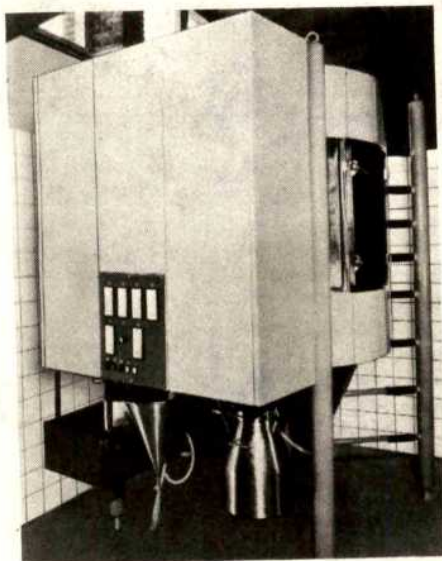
### Stanton Redcroft

Stanton Redcroft will be exhibiting a new simultaneous thermobalance-differential thermal analyser for measuring weight and thermal changes of materials. This equipment uses micro-processors and allows the physical and chemical phenomena of many different materials to be studied. The established universal digital temperature programmers will be displayed showing their versatility in operation from desktop calculators. Also on show will be a well established range of thermobalances and differential thermal analysers. Of particular note is the quality control application of the TG 750 in fields such as plastics and food. The flammability field is now rapidly growing, and the complete range of oxygen index test equipment operating at room temperature and up to 400 °C will be displayed.



Other products manufactured by Stanton Redcroft include a hot stage microscope attachment, thermomechanical analyser and mineral insulated thermocouples.

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Anhydro pilot spin-dryer

#### Anhydro

Equipment on show on the Anhydro stand includes a new laboratory spin flash drying plant suitable for tests with highly viscous materials such as pastes. Also available is a new compact spray dryer for use as a pilot system.

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#### Caldyn

In Caldyn gas impregnation and atomising plants the property of gas/liquid mixtures to have a substantially lower sonic velocity than the gas or liquid alone is utilised to atomise liquids and to generate minute gas bubbles. Liquid and gas are mixed together in the Caldyn CSL nozzle and accelerated to the sonic (critical) velocity of the two-phase mixture. The mixture is expanded to the environmental pressure at the nozzle outlet. Atomisation is achieved with very low energy consumption.

Circle No. 96 on Reader Enquiry Card.

#### Langley Alloys

In addition to their general range of special alloy materials, stainless steels, nickel alloys, cupro-nickel and aluminium bronze, and valves in stainless steel and nickel alloys, special features on the Langley Alloys stand include: Ferralium alloy 255—demonstration of its pitting resistance and crevice corrosion resistance. Ferralium alloy 288—new cast alloy with improved resistance to phosphoric and sulphuric acids. A new cast nickel-molybdenum alloy, Niralium alloy 130, is introduced.

Circle No. 95 on Reader Enquiry Card.

#### V. C. Filters

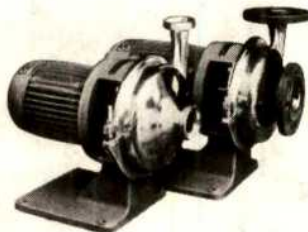
V. C. Filters, in association with L. B. Holliday & Co. of Huddersfield, have developed and are now marketing multiple cell electrolyzers for use in electrochemical processing. These units are suitable for a variety of electrochemical processes including electro-organic synthesis, 'chlor-alkali' processes such as chlorine/caustic soda, sodium hypochlorite, chlorate and bromate manufacture and the re-oxidation of many inorganic redox species used for oxidation of organic compounds. The electrolyzer contains 1m<sup>2</sup> electrodes and can be readily built up to a maximum of 40 cells per unit. The assembly may be of mono-polar or bipolar arrangement and contains optional ion exchange membranes. Other sizes of electrolyzer are also available, including a small laboratory unit which is completely self contained and can be supplied with various interchangeable electrode materials. The unit can be extended to operate in a bipolar mode by the inclusion of more cells. An intermediate or pilot sized plant is also available.

Circle No. 94 on Reader Enquiry Card.

#### Driam

Driacoater filmcoating equipment from Driam is designed for filmcoating pharmaceutical tablets, hard and soft granulates and pellets with organic solvents and aqueous dispersions. A fluidised bed is developed in the drum of the Driacoater by drying air entering over a rotating distributor by way of hollow ribs immersing into the product bed. By elimination of baffles and development of a fluidised bed, an even mixture, a homogeneous coat as well as a gentle handling, also of very sensitive products, can be achieved. At the same time the counter-current air flow guarantees efficient drying.

Circle No. 93 on Reader Enquiry Card.



Hilge Hygia-Bloc pump

#### Hilge

Hilge are showing a wide range of pumps made of chromium nickel steel for applications in chemical and biochemical engineering, water- and industrial techniques, surface-, airconditioning- and cleaning-treatments.

Circle No. 92 on Reader Enquiry Card.



## Alfa's new 1979-80 Catalog

### NOW WITH ORGANICS.

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Company: \_\_\_\_\_  
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Circle No. 19 on Reader Enquiry Card.





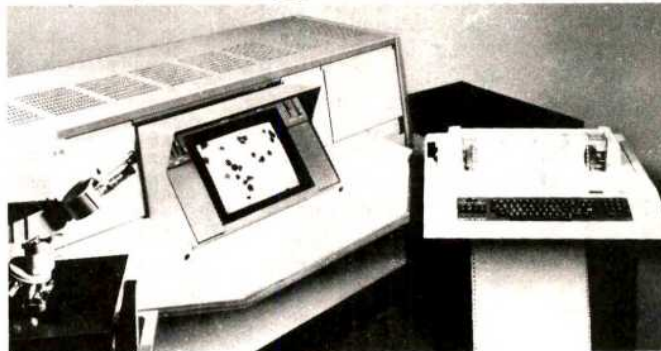
# The programmable computerised image analyser that doesn't need a programmer

You can program the new Quantimet System 23 for your image analysis applications with no computer programming knowledge.

Image analysers count, measure and classify features in images from optical and electron microscopes, photographs, negatives, cine film or macro objects. Applications vary widely and virtually each one requires a different type of analysis routine.

Unlike any other image analyser, the new System 23 incorporates a unique operator-interactive image analysis keyboard which enables you to program the System 23 for your image analysis application **without** using FORTRAN, BASIC or any other exotic programming language.

For example, when analysing features such as particles, fibres, etc., depressing the keys **MEASURE FEATURE COUNT DISTRIBUTED BY**

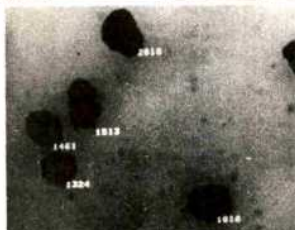


**LENGTH** results in a frequency distribution of feature length which is displayed on the system monitor as illustrated in the histogram below.

Using a similar sequence of keyboard instructions, the operator can enter experimental variables and compose mathematical expressions such as quadratic equations, derivatives and compound descriptions of feature shape and texture.

Now you can easily develop, test and enter new measurement concepts and store immediate results on the Quantimet monitor or printout. After you have written your analysis routine, store it on a high

speed magnetic disc for future use. When repeating the analysis, insert the disc in the computer, enter the routine number and the System 23 runs automatically. **FILL IN THE COUPON BELOW AND MAIL IT NOW - OR CALL US ON THE PHONE.**



Feature Parameters displayed at each feature assist in analysis of specimen.



Intermediate or final Histograms displayed on monitor. Histograms can be logarithmic, differential or integral.



Image Editor Pen (optional) used on problem specimens to ACCEPT, REJECT, CUT, JOIN or FILL.

## Cambridge Scientific Instruments Limited

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**U.S.A.** 40 Robert Pitt Drive, Monsey, New York 10952, tel. (914) 356 3331 telex 137 305  
**Canada** 2710 Brabant Marineau, Montreal, Quebec H4S 1L1, tel. (514) 337 4343  
**W. Germany** Harnackstrasse 35-43, D4600 Dortmund 1, tel. (0231) 12 60 86 telex 08227346  
**France** Centre d'Affaires Paris Nord, 93153 Le Blanc Mesnil, tel. 931 01 34 telex 230185  
**S. Eastern Europe** Lainzer Strasse 90, A-1130 Vienna, Austria, tel. 82 25 55 telex 13426

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Please send me details of the Quantimet 720, System 23

Name .....

Company .....

Position .....

Address .....

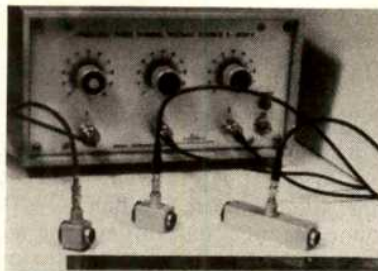


**Charles Austen Pumps Ltd**

Charles Austen are exhibiting a comprehensive range of gas, air, vacuum and liquid pumps. These purpose-designed pumps are of small physical size, quiet in operation and simple to service. They are of the membrane type and completely oil free. Flow rates are 0.70 litres per min, pressure  $0.4 \text{ kg cm}^{-2}$  and vacuum  $0.740 \text{ mm Hg}$ . The pumps are used for medical and industrial research purposes, process control instrumentation and in laboratory instruments, for example flame photometers, spectrophotometers, gas and air sampling instruments, environmental chambers, vacuum embedding instruments and ageing ovens. Charles Austen diaphragm liquid pumps are self priming and ideally suited to applications where scavenging is necessary. The range of small centrifugal liquid pumps are used for filtration duties, cooling units and X-ray processing machines. The pumps are used in the following instruments manufactured by Charles Austen: the Vacuum Tweezer, which provides an effective and safe means of manipulating micro-miniature or delicate components during examination or assembly and a completely portable atmosphere sampling unit, the FI Mk. 1, incorporating battery mains charger, flowmeter with control valve and timer. **Circle No. 91 on Reader Enquiry Card.**

**Physik Instrumente**

Smallest movements of less than  $0.01 \mu\text{m}$ , can be produced with piezoelectric translators manufactured by Physik Instrumente. The translators feature high positioning accuracy with a resolution of  $0.005 \mu\text{m V}^{-1}$ ; continuously variable length setting from 0 to  $40 \mu\text{m}$  per piezoelectric unit; modular construction in small units which can be combined easily with various positioning components; and fast reaction to the applied voltage with the total expansion taking place in microseconds.



Precision will not change during the lifetime of the devices due to the absence of wearing parts, and remote control makes them particularly suitable for use in automatic process control systems. Shown above are three translators being run from a Physik three-channel voltage source. **Circle No. 90 on Reader Enquiry Card.**

**Neptune**

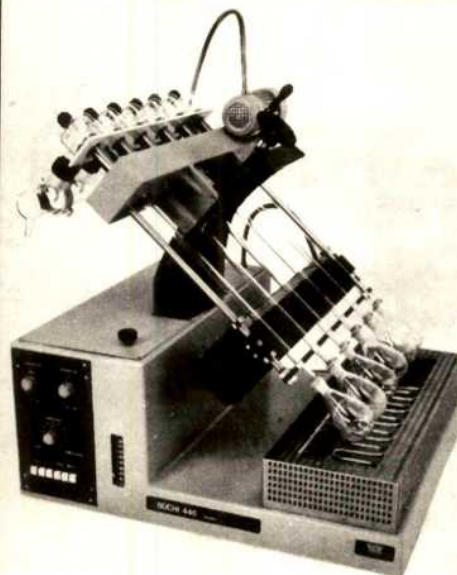
THE Neptune European Group are exhibiting a comprehensive range of their products together with models and photographs. The principal exhibit is the Autopress, a practical and mechanically uncomplicated automatic filter press. Developed by Johnson-Progress and Moseley Rubber Company, the Autopress has a total cake discharge (press open) time of less than 3 min. Membrane Moseley plates provide filtration at up to  $15 \text{ kg per cm}^2$  and squeeze pressures of up to  $20 \text{ kg per cm}^2$ . Air-assisted cake discharge permits automation of the total press sequencing. Light safety curtains are eliminated and there are no moving parts in contact with the process liquids. Other exhibits include Glenfield industrial gate valves, the EPEX air relief valve for effluent systems, and the Neptune range of flowmeters and Tricon control instrumentation. The Neptune companies, OCP (Belgium), Hydrotec (France) and Kary (FRG), are specialists in water, waste water and sludge treatment and on exhibition will be tube settling modules, a mixed media column and a comprehensive photographic and diagrammatic display of their capabilities in the fields of Microfloc and water and waste water treatment technology. **Circle No. 89 on Reader Enquiry Card.**

**System for trace elements analyses**

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**Pretema**

The 4000 seria is Pretema's third generation colour measurement system. The spectrophotometer itself satisfies all requirements of a modern spectrophotometer as regards optics, light sources, accuracy of measurement, speed, design, comfort in operation and maintenance. Matched with modern computers, with external data and program storage and input/output devices unreached in speed and comfort, and with a colour software that has grown in a 20 years' search for the best assistance to the colourist, makes it an extremely attractive system.

**Circle No. 88 on Reader Enquiry Card.**

**Huyck Fez**

The complete range of Fez woven and needled filter media will be shown atACHEMA 79. Filter fabrics for liquid filtration include special fabrics for use in filter presses, rotary drum filters or as made-up bags for candle filters. Also on show is the first fibreglass needle felt with fibreglass base fabric—Huyglas—this new filter medium will have wide application in hot gas filtration. Huyglas is designed for application in pulse jet filters and also features higher mechanical stability than has previously been achieved with glassfibre woven filter media.

**Circle No. 87 on Reader Enquiry Card.**

**Du Pont**

Chemical industry applications illustrating the total corrosion protection afforded by Du Pont's fluoroplastic and fluorinated elastomeric materials will be the feature of the company's stand atACHEMA. Most types of fluid handling equipment are now available with Teflon linings. In addition, diaphragm valves lined with thermoplastic Tefzel fluoropolymer, a material offering chemical resistance close to that of Teflon, have also come on the market. A new type of Du Pont heat exchanger known as the 'interchanger' will be exhibited atACHEMA for the first time. This development of the shell-and-tube concept takes double advantage of the chemical resistance of Teflon. One corrosive medium, such as spent sulphuric acid or phosphoric acid, flows through the tubes of Teflon, while another corrosive fluid can flow around the tubes and through the shell, which is also Teflon-lined. Heat can be transferred from one liquid to the other in either direction. Seals and similar components made of Kalrez perfluorinated elastomer will also be on show. This material combines many of the elastomeric properties of Viton with thermal stability, chemical resistance and electrical characteristics like those of Teflon.

**Circle No. 86 on Reader Enquiry Card.**

**Mastermix Engineering**

The Mastermix Mastermill is a horizontal bead mill for the high quality super fine dispersion of paints, inks, plastisols, organosols etc. It is capable of handling high viscosity materials with ease. Two versions will be on show, a 15 litre model with throughput capacity of 60 to 300 litres per h and the newly introduced 1 litre version which has been specially designed for experimental and pre-production batch work. The Mastermix Mastermill is now available in capacities of 1, 15, 30, 60 and 150 litres.

**Circle No. 85 on Reader Enquiry Card.**

**Faudi**

Standard Faudi pressure filters offer versatile combinations for applications in chemical and petrochemical processes, in the mineral oil industry, in metal working, electroplating and for corrosive, toxic, inflammable and other hazardous liquids as well as for gases, air and steam. The range features 8 sizes, 8 filter elements, 3 pressure ranges, 4 cover closures and 2 materials. A comprehensive selection of filter ratings between 1 and 1,000  $\mu\text{m}$  can be combined to cater for a wide variety of requirements. The filter elements are interchangeable and can be fully dismantled for easy cleaning.

**Circle No. 84 on Reader Enquiry Card.**

# ANIMAL OPERATING TABLE

- Sterilisable & easily cleaned
- Sturdy but lightweight for effortless handling
- Versatile design
  - table top quickly removed for use without legs
  - height and tilt adjustment for operating comfort
  - suitable for most animal sizes
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Plus a full range of sterilisable stainless steel accessories, cleats and animal support plates. Send for details today.



**NOTE:** To keep animals warm while on the operating table we offer our Homeothermic Blanket System. Details on request.

A four-way electrical socket is fitted to one leg (home market only) plus an earth point on the underside of the table.

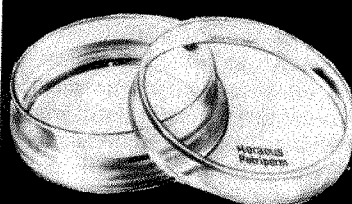
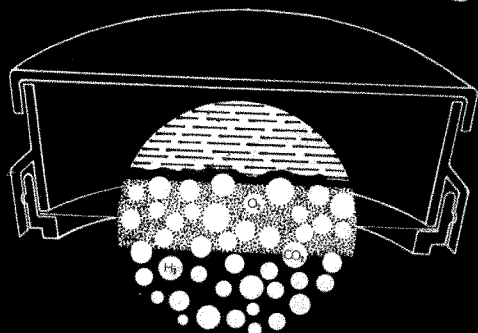
**BioScience** (incorporating C.F. Palmer and George Washington Ltd.)  
Harbour Estate, Sheerness, Kent ME12 1RZ.  
Telephone: Sheerness (079-56) 67551 Telex: 965088



**Circle No. 18 on Reader Enquiry Card.**



## A new approach in tissue culture



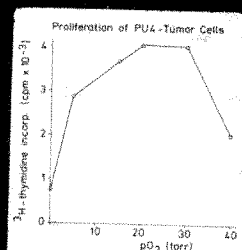
### PETRIPERM

a novel culture dish  
with gas permeable  
membrane as cellular  
support.

Additional advantages of the PETRIPERM membrane are:

- UV permeable 200 nm
- Suitable for high power light and fluorescence microscopy
- The membrane can be cut for cell cloning, electron microscopy, staining and documentation
- Chemically resistant to acids, bases and organic solvents.

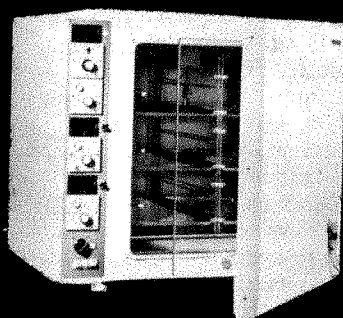
PETRIPERM is available either with a hydrophilic surface for optimal attachment, or with a hydrophobic surface for easy detachment of adherent cells.



In the intact animal, the pH and the partial pressure of both oxygen and CO<sub>2</sub> of the cell environment are under total biochemical and physiological control. This state is not even approximated by available techniques of monolayer cell culture in conventional tissue culture ware. Continuously changing gradients of these parameters in the micro-environment of cultured cells will obscure the biological significance of culture dependent phenomena. PETRIPERM is the prerequisite for the precise control of pO<sub>2</sub>, pCO<sub>2</sub> and pH in the cellular environment as gases reach the cultured cells directly through the highly gas permeable 25 µm thick membrane.

### Heraeus— CO<sub>2</sub>-Incubator B 5060 EK/CO<sub>2</sub>

We build the  
incubator with  
appropriate  
pCO<sub>2</sub>, pH and pO<sub>2</sub>  
control units



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# Heraeus

Circle No. 15 on Reader Enquiry Card.

## The Pendulum and Toxic Cloud

The Course of Dioxin Contamination

Thomas Whiteside

This powerful investigative report raises grave questions about the long term effects of low-level contamination of the environment by very highly toxic substances, and describes the dilatory behaviour of the United States government in taking appropriate regulatory action against such hazards, especially in view of the results of such appalling accidents as Seveso, and the defoliation of Vietnam. Simultaneous publication Cloth £10.80 Paper £3.60.

## The Kindly Fruits of the Earth

An Environmental Study of an Embryo Ecologist

G. Evelyn Hutchinson

In this charming memoir, a founder of population ecology describes his childhood, boyhood and early youth in Cambridge at a time when important discoveries and debates in science were made. This book reveals, in a hundred ways, the distinguishing feature of Hutchinson's mind: an all-embracing curiosity that moves between the scientific and the everyday in a way that illuminates both. Forthcoming £13.30

## An Introduction to Population Ecology

G. Evelyn Hutchinson

This introduction is written to appeal not only to beginners in ecology but also to an occasional historian, economist or sociologist, and those biologists in other areas of the science. £12.60

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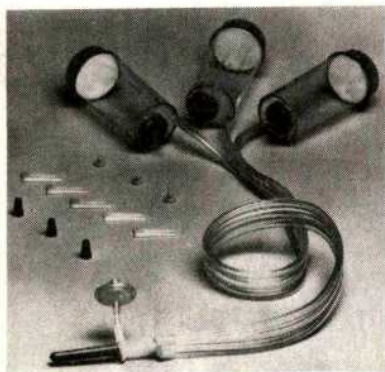
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(In Massachusetts and International: 617-482-9595)

NEN Chemicals GmbH, Dreieich, W. Germany; NEN Canada Ltd., Lachine, Quebec

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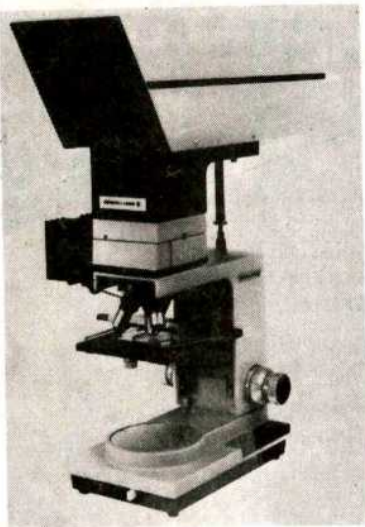


The Millipore Steritest system

### Millipore

Millipore will present at ACHEMA a comprehensive range of new, application-oriented displays in the processing, analytical and research fields. You can see at ACHEMA 79, Millipore products such as the industrial cartridge-filter series, including the new Durapore and Fluorex and Fluorogard. Millipore water purification systems combine reverse osmosis, microfiltration and ultrafiltration equipment to meet laboratory and process requirements for purified and ultrapure waters. A new 'closed system' concept for sterility testing will also be on show together with other Millipore products for particulate and microbiological analysis, ultrafiltration and fluid sterilisation.

Circle No. 83 on Reader Enquiry Card.



Bausch &amp; Lomb direct-view microscope

### Bausch & Lomb

The Scientific Optical Products Division of Bausch & Lomb are displaying the complete range of Balplan and StereoZoom microscopes together with the latest developments in microscope accessories including photographic equipment.

Circle No. 82 on Reader Enquiry Card.

### Hick Hargreaves & Co.

A new, skid-mounted Hick Sutorbilt blower package unit (capacities up to  $2,000 \text{ m}^3 \text{ h}^{-1}$ ) is exhibited by Hick Hargreaves. The package includes blower, motor and belt drive, inlet and outlet silencers, and optional relief valve and acoustic enclosure. The advantages offered are low price, quick delivery and easy installation. Also on display is a mimic diagram incorporating various vacuum raising products, heat exchangers and a fatty acid scrubber used on deodoriser systems. Energy conservation is demonstrated here by the recovery of heat absorbed by the cooling water in a heat exchanger and its conversion in a 'flash' vessel to low pressure heating steam.

Circle No. 81 on Reader Enquiry Card.

### G. H. Zeal Ltd

G. H. Zeal Ltd of London, makers of high quality temperature measuring, recording and controlling instruments, will be displaying a large selection of their extensive product range at ACHEMA. The emphasis of the display will be on the very popular groups of bi-metal, vapour pressure and mercury in steel actuated dial thermometers, both the new stainless steel cased heavy duty range and the 'Slim-line' series. Mercury in steel actuated chart temperature recorders will also be featured along with thermometers fitted with electric contacts and a working model of the Zeatron remote reading electronic thermometer. The recently introduced Zeatron single channel push button operated portable electronic thermometer will also be on show. The display will include a large selection of both glass and metal hydrometers, mercury and spirit filled laboratory thermometers and meteorological thermometers.

Circle No. 80 on Reader Enquiry Card.

### Newman Hattersley International

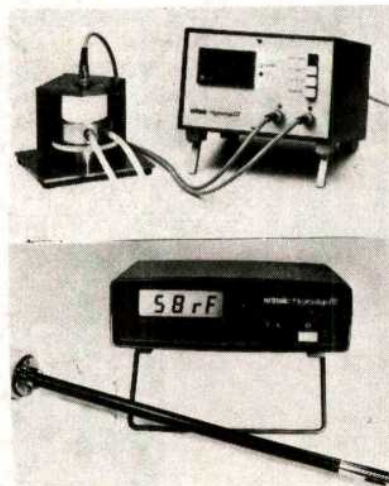
On the Newman Hattersley stand will be a selection of cast, forged and stainless steel valves, particularly suited to the European chemical industry. These include Heaton cast and forged steel gate, globe and check valves, Daytona butterfly valves and the internationally approved firesafe Heaton carbon and stainless steel ball valve. Also on display will be the Hender Bellows sealed valve which has demonstrated its atmospheric leakproof characteristics in a variety of tough environment applications. Also on the stand, Sydney Smith Dennis Ltd. will be displaying a wide range of pressure gauges in a variety of sizes and case materials to suit the varying needs of the chemical industry.

Circle No. 79 on Reader Enquiry Card.

### Volac

On view at ACHEMA is the full range of Volac volumetric glassware, disposable Pasteur and serological pipettes, Polystop reagent, dispensing and dropping bottles, and the Volac pipette plugging and de-plugging machine. John Poulten Ltd. will be introducing a range of pipetting devices, new bottles for water sampling procedures and individually packed sterilised disposable pipettes. The new Volac Micro-Pipettor is produced in capacity ranges  $0-50 \mu\text{l}$ ,  $0-100 \mu\text{l}$ ,  $0-250 \mu\text{l}$  and  $0-500 \mu\text{l}$ , each colour coded and fully adjustable in  $0.2\%$  increments, with digital capacity indication. Mk. II Volac pipette controllers feature graduated plungers, free flow control, universal push-fitting pipette attachment accepting all pipettes up to  $10.3 \text{ mm}$  diameter and robust autoclavable construction. The  $0-2 \text{ ml}$  model is an ideal instrument for use with all sizes of Pasteur pipettes. A complete range of sterilised, disposable graduated pipettes is available in six capacities from  $1 \text{ ml}$  all colour coded, supplied in sleeves of five, each pipette sealed within its own individual compartment.

Circle No. 78 on Reader Enquiry Card.



Retronic Hygroskop DT with a WA measuring station (top) and Hygroskop BT with a KG-HT1 probe (bottom)

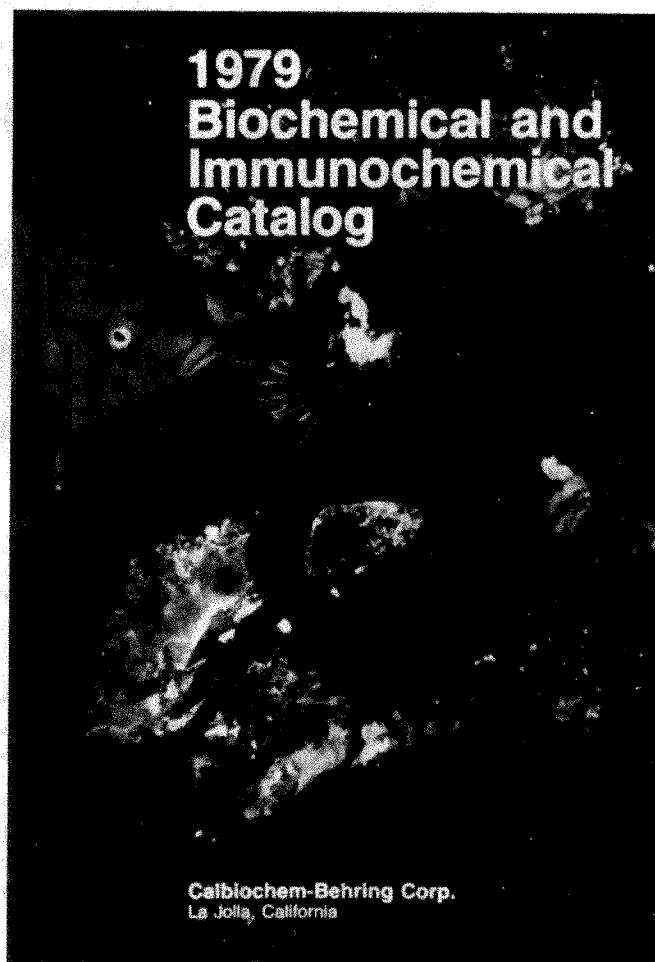
### Rotronic

The new Rotronic Hygroskop HC humidity controller instantly detects any change in relative humidity with the reliability and accuracy indispensable to industrial applications. The sensing element used with this controller is highly stable and gives excellent precision and high accuracy. Two relay outputs, each with make and rest contacts, operate at two different humidity levels. These levels are independent and can be easily adjusted with the help of a linear voltage output. Both the controller and its probe require little maintenance. The Rotronic Hygroskop DT high-precision laboratory humidity measuring apparatus is available as



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two different models. The low humidity model now permits measurements between 0 and 50% RH with a precision of  $\pm 0.5\%$  RH between 0 and 10% RH, and  $\pm 2\%$  for higher humidity. The standard model takes measurements at 10–100% RH with an accuracy of  $\pm 2\%$  RH. The Hygro-skop BT is a portable electronic humidity meter which offers easy calibration and precision of  $\pm 1.5\%$  RH.  
**Circle No. 77 on Reader Enquiry Card.**

### Geho Pompen

Holthuis of Holland are represented atACHEMA '79 through their subsidiary company Geho Pompen. The company design and manufacture piston and diaphragm pumps for the feeding of filter presses, incinerators, heat treatment sludge plants, solids transportation, etc. Flow control systems designed by Holthuis permit a reduction of cycling time to 10–30% for feeding filter presses when compared with conventional diaphragm pumps. Geho pumps also allow the transportation of de-watered filter cake from centrifuges, belt presses, and so on. Further new developments are special pumps for the hydraulic transportation over large distances of material such as ore concentrates, coal slurries and industrial wastes. New developments in the field of hydraulic rapid closure systems are also shown.

**Circle No. 76 on Reader Enquiry Card.**

### Wild Heerbrugg

New products on show include the M5APO stereomicroscope which achieves the highest standards of sharpness, contrast and orthochromatism. The wide range of accessories made for the M5A will also suit the high-performance M5APO. Also on show the MPS 45 Photoautomat for macrography and photomicrography and the M520 IR image converter which provides binocular observation in the near-IR range. The new Wild Heerbrugg EB transmitted-light stand provides better conditions for the observation and photography of specimens in the transmitted light bright field and with polarisation.

**Circle No. 68 on Reader Enquiry Card.**

### Nova Werke

Nova present two new ranges of high-pressure laboratory units. (1) A mobile unit for use with liquids and gases at pressures of up to 700 bar and at temperatures of up to 350 °C. A new feature of particular interest is an electronically controlled stirring unit, the Nova Rotacontrol. (2) The new Novawiss high pressure 'equiphase' research unit has been designed specifically for visual observation of reactions under a pressure regime of up to 7,000 bar and at up to 450 K.

**Circle No. 75 on Reader Enquiry Card.**

### Polytec

Polytec have specialised in the laser field since its foundation and have produced He-Ne-lasers since 1974. The new Polytec range is made up of cylindrical laser heads all having hard sealed integral optics for long shelf life. Units from 0.5 mW to 1.5 mW have the choice of integral or separate power supplies. The model 605 with a 0.5 mW multimode output is only 40 mm in diameter and 265 mm long including power supply making it the smallest commercially available He-Ne laser.



The compact Polytec PL 605 laser

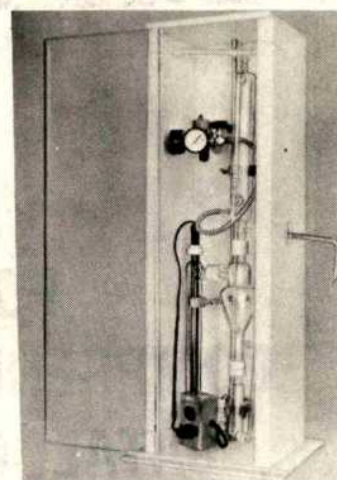
The Karlsruhe system is designed for quick determination of the distribution of particle size and particle velocity in flows of aerosols and in translucent liquids at high particle concentrations (over  $10^3$  particles per  $\text{cm}^3$ , depending on particle size). It is an ideal instrument for use in the laboratory and production: as a fast analyser for dust or power particle size distributions in dispersed state; for gas bubble distributions in transparent liquids; for periodic or sampling quality control; and as a fast measuring instrument when working with dispersed phases in gaseous or liquid flows. The modular electronic system comprises interfaces to computer, teletype, displays, tape punch and x-y plotters. The Laser-2-Focus-Velocimeter from Polytec is a compact electro-optical instrument for the precise non-intrusive, two-dimensional measurement of instantaneous velocity, flow direction and turbulence in liquid and gas flows. The velocity range is 1–2,000  $\text{m s}^{-1}$ . The instrument measures the transient time of small particles which are dispersed in the fluid between two focused laser beams. The velocimeter is not affected by background illumination. This results in high sensitivity even under adverse conditions.

**Circle No. 74 on Reader Enquiry Card.**

### Verder

Thirty-five different pumps are now available from Verder (Deutschland) GmbH with capacities of between 0.0008 ml per min and 700 litres per hour. The new tube-type models are particularly suitable for use in liquid chromatography and in other applications requiring continuously varying liquid feeds. A new micropump model will also be introduced at theACHEMA show.

**Circle No. 73 on Reader Enquiry Card.**



Gilmont Model V water still

### Roger Gilmont Instruments

The Gilmont Instruments Model V water still delivers a product with the ultimate purity of up to 95% conductivity water quality from tap water feed. It produces this quality product at a volume of 1.5 liters per hour continuously. The Model V plugs into an ordinary 115-V a.c. receptacle, and uses a 1,000-W Vycor heater at 90% heating efficiency. An all-acrylic housing offers rigidity and protection to the all glass still. When housed, it can be used on a rolling cart, or bench mounted for fixed operation. Assembly of the still inside the housing is fast and uncomplicated due to pre-set clamp holders, Teflon sleeves and Taper-Tite nuts.

**Circle No. 72 on Reader Enquiry Card.**

### Symalit

The new Du Pont line of chemical vessel linings, Armalon, is introduced onto the European market by Symalit of Lenzburg, Switzerland. Armalon lining laminates consist of glass fabric bonded to a layer of Teflon film. The film is inert to virtually all chemicals and corrosives from  $-240\text{ }^{\circ}\text{C}$  to  $220\text{ }^{\circ}\text{C}$ . The laminates are highly resistant to de-lamination and conform well to vessel shapes. They will accept epoxy, polyester, thermo-setting and some general-purpose adhesives to form strong long-lasting bonds on existing metal vessels.

**Circle No. 71 on Reader Enquiry Card.**

### Degussa

This stand features chemicals for environmental protection (hydrogen peroxide, sodium chlorite and other oxidation chemicals, TMT 15). Catalysts made by Degussa include activated nickel; precious and non-precious metal carriers and vehicle exhaust gas catalysts. Degussa show a full range of precious metals, special metals and alloys, electrical thermometers and tantalum coatings.

**Circle No. 70 on Reader Enquiry Card.**



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### Director Chemical Research Ann Arbor, Michigan

The Pharmaceutical Research Division of Warner-Lambert is seeking an outstanding medicinal chemist to head its Department of Chemistry and lead a large team of organic, physical and analytical chemists in the exciting and demanding job of finding new and effective medicines.

Current emphasis is in the areas of Anti-infective, CNS, Cardiovascular, Anti-allergic and Immunoregulatory drugs.

The research facilities located in Ann Arbor, Michigan, are among the finest and best equipped in the world.

The successful applicant will have:

- An outstanding record of achievement in some area of medicinal chemistry, particularly in relation to the discovery of new drugs.
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- Management and leadership skills, with abilities to encourage innovation and motivate the staff to a high level of performance.

and be responsible for:

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- Some large scale preparations.

The Director of Chemistry will report to the Vice President of Preclinical Research and work closely with the Director of Pharmacology and the Director of Toxicology as well as members of the Clinical Research Group.

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WARNER-LAMBERT RESEARCH LABS  
2800 Plymouth Road  
Ann Arbor, Michigan 48106 U.S.A.

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W165(A)



## Warner-Lambert

### UNIVERSITY OF NOTTINGHAM SCHOOL OF AGRICULTURE Sutton Bonington DEMONSTRATOR IN AGRICULTURE

Applications are invited from candidates with good honours degrees in Agriculture or appropriate Agricultural Sciences (including those who will graduate in 1979) for the post of Demonstrator in Agriculture to assist with laboratory and field classes in Crop Production. The appointment will be for a period of three years commencing September 1979 and there will be an opportunity to undertake research for submission for a higher degree.

Salary within the range £3,689 to £4,232 per annum (under review).

Further details and forms of application, returnable not later than July 6, 1979, from the Staff Appointments Officer, University of Nottingham, University Park, Nottingham NG7 2RD. Ref: 699. 2157(A)

### UNIVERSITY OF CAMBRIDGE ANIMAL PRODUCTIVITY/ NUTRITION Applications are invited for a UNIVERSITY DEMONSTRATORSHIP

in Animal Productivity/Nutrition in the Department of Applied Biology. Qualifications—good Honours degree, research experience in some aspect of animal productivity related to nutrition and competence in biometry.

Salary in scale £4,505 to £5,591. Pensionable under U.S.S. Limited contribution to removal expenses.

Further details from and applications including full personal particulars, list of publications and names and addresses of not more than three referees to the Secretary, Faculty of Biology 'A' Appointments Committee, Department of Applied Biology, Pembroke Street, Cambridge CB2 3DX, not later than July 6, 1979. 2159(A)

### UNIVERSITY OF LONDON INSTITUTE OF NEUROLOGY Applications are invited for TWO RESEARCH TECHNICIAN POSTS

in a group supported by a 5-year M.R.C. grant and working on the role of 5HT in the brain. Experience in neurochemistry, pharmacology or the study of behaviour is an advantage, but not essential.

Salary £2,915 to £3,779 (including London Weighting).

Curriculum vitae and names of two referees to Professor G. Curzon, Department of Neurochemistry, 33 Johns Mews, London WC1N 2NS. 2087(A)

### UNIVERSITY OF CAMBRIDGE DEPARTMENT OF CLINICAL BIOCHEMISTRY School of Clinical Medicine Hills Road A GRADUATE

#### RESEARCH ASSISTANTS

is available in the above Department from August 1, 1979. The post is supported by a grant from the Wellcome Trust and is funded September 1981 with a possible extension for a further two years.

The starting salary is £3,961.

The project involves the purification and immunoassay of human specific proteins and the application of such measurements to clinical problems.

For further details contact Prof. C. N. Hales, Department of Clinical Biochemistry, University of Cambridge Clinical School, Addenbrooke's Hospital, Hills Road, Cambridge.

Closing date for application, July 2160

### THE HANNAH RESEARCH INSTITUTE (for studies relating to the production and utilisation of NUTRITIONIST

There is a vacancy in the Nutrition and Metabolism Section of the Animal Studies Department for a person to undertake work on the energy metabolism of farm animals. The successful applicant will take charge of animal calorimeters and will be responsible for the conduct of section's calorimetric experiments.

The appointment will be made at Scientific Officer level, for minimum qualifications are a 1st in an appropriate subject (Agriculture, Nutrition, Agricultural Science, Biology, Biochemistry or Chemistry H.N.C. with appropriate experience).

The salary (under review) is £4,415, and there is a contributory pension scheme.

Requests for further details should be addressed to:

The Secretary,  
The Hannah Research Institute,  
Ayr KA6 5HL.

Applications close on July 6, Ref: HRI 33. 2162

### PRE AND POSTDOCTORAL POSITIONS

are available for individuals with microbiology background for studying the protein synthesising system with special emphasis on ribosome biogenesis, biogenesis and function. Teaching opportunities are available. Reply with résumé to: Dr Bruce Sells, Professor and Director, Laboratories of Molecular Biology, Faculty of Medicine, Health Sciences Centre, Memorial University of Newfoundland, St John's, Newfoundland A1B 3X6, Canada. W163



**UNIVERSITY OF  
LIVERPOOL  
DEPARTMENT OF  
OCEANOGRAPHY  
POSTDOCTORAL SENIOR  
RESEARCH ASSISTANT**

Applications are invited for the post of Senior Research Assistant in connection with a survey of trace metals to be carried out in the Mersey estuary. Candidates should possess a Ph.D. in analytical chemistry, marine chemistry, or a related subject. The project is sponsored for three years by the North West Water Authority. The initial salary will be either £4,232 or £4,505 per annum. Applications, together with the names of three referees, should be received not later than July 7, 1979, by the Registrar, The University, P.O. Box 147, Liverpool, L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/635/N. 2104(A)

**UNIVERSITY OF  
LIVERPOOL  
DEPARTMENT OF  
BIOCHEMISTRY  
RESEARCH ASSISTANTS**

Applications are invited from graduates in Biochemistry or related subjects to work on projects investigating (a) the turnover of specific proteins normal and abnormal muscle. The project is funded for three years by the MRC; and (b) the regulation of protein breakdown in muscular dystrophy. The project is funded for three years by the Muscular Dystrophy Group of Great Britain. The posts are under the supervision of Dr. R. J. Beynon and applicants with an upper second or first class honours degree will be considered as candidates for a Ph.D. The posts are tenable from October 1979 at an initial salary of £3,689 per annum. Applications, together with the names of three referees should be received not later than July 6, 1979, by the Registrar, The University, P.O. Box 147, Liverpool, L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/639 (a) or /N. 2106(A)

**UNIVERSITY OF  
EDINBURGH  
TECHNICIAN**

Required for the DEPARTMENT OF GENETICS, Kings Buildings, to join a team working on the cellular molecular biology of eye tissues. Experience in at least one of the following techniques required: cell culture, molecular biology, enzyme assays, electrophoretic separation techniques. Further training will be given if needed. This appointment is supported by the Medical Research Council and will be for a period of 3 years. Salary on the scale £3,474 to £5,616 p.a. Applications should include names and addresses of two referees. REF: NS202. Application forms for the above post can be obtained from the Personnel Office, University of Edinburgh, 63 Leith Bridge, Edinburgh EH1 1LS. 031-556-2930. 2119(A)

**LEGUME AGRONOMIST/  
BACTERIOLOGIST**

to participate in international field experiments to assess yield response of legume crops to inoculation with *Rhizobium*. Ph.D. in legume agronomy, physiology or bacteriology required. Must be prepared to travel extensively and have previous field experience with tropical legumes, and legume inoculants. Demonstrated ability to work on multi disciplinary projects would be an advantage. Application to: Dr. Jakek Halliday, NITFAL, c/o University of Hawaii, P.O. Box 100, Paia, Hawaii 96779. The University of Hawaii is an EEO/AA Employer. W159(A)

**ROTHAMSTED  
EXPERIMENTAL STATION  
Harpden, Herts. AL5 2JQ  
MOLECULAR BIOLOGIST**

There is a vacancy for a molecular biologist with experience in nucleic acid technique to join a team in the Biochemistry Department working on the genes specifying the cereal storage proteins. This team is part of a new programme within the Agricultural Research Service concerned with understanding and manipulating of plant genes.

Qualifications: First or Upper Second Class Honours Degree in Biochemistry or related subject with a minimum of two years' relevant postgraduate research experience. Preference given to candidate with a Ph.D. degree. The appointment will be for three years in the first instance.

Appointment in grade of Higher Scientific Officer (£4,101 to £5,448. (Pay Award pending.) Non-contributory superannuation.

Apply in writing to the Secretary, giving names and addresses of two referees and quoting Ref. 366 by June 29, 1979. Further details on request. 2114(A)

**ROTHAMSTED  
EXPERIMENTAL STATION  
Harpden, Herts. AL5 2JQ**

STATISTICIAN required to work on problems of applied multivariate statistics. The successful candidate will be expected to assist in developing new methodology, to recognise new problems and to apply newly-developed methods, as well as well-established ones, to the analysis of multivariate data. A high level of research ability is sought. Applications will necessitate the use of computers, often using the Genstat statistical package, but will also require the writing of new programs, for which a sound knowledge of modern numerical techniques is essential. The post involves some consulting work, especially with soil scientists.

Minimum qualifications: 1st or upper 2nd class honours degree in mathematics or statistics, and preferably a higher degree or equivalent post-graduate experience in a relevant field. An interest in applied multivariate analysis is desirable.

Appointment in grade of Scientific Officer (£2,839 to £4,415) or Higher Scientific Officer (£4,101 to £5,448) (Pay Award pending). At least two years' relevant post qualifying experience is required for appointment as HSO. Non-contributory superannuation.

Apply in writing to the Secretary, giving names and addresses of two referees and quoting Ref. 397 by June 29, 1979. Further details on request. 2115(A)

**THE LONDON HOSPITAL  
MEDICAL COLLEGE  
(University of London)  
DEPARTMENT OF  
HAEMATOLOGY**

Applications are invited for the post of

**TECHNICAL ASSISTANT**

in the Serology Unit of the Department of Haematology. Candidates should hold appropriate University or I.L.M.S. qualifications or equivalent.

The post comprises forensic serology, both paternity tests and investigation of stains.

Previous experience in blood group serology and/or electrophoretic techniques preferred.

Salary according to age and experience on Whitley Council Scale within the range £3,615 to £6,123 per annum, including London Weighting (currently under review). Four weeks' annual holiday, plus extra days when College is closed.

Further details from Professor Barbara Dodd, 01-247 5454, ext. 360.

Applications in writing to The Secretary, The London Hospital Medical College, Turner Street, London E1 2AD, quoting Reference No. SUH6/79. 2095(A)

**CSIRO AUSTRALIA**  
**Division of Materials  
Science Production  
Technology Laboratory  
Woodville South Australia**

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees—2,400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

**General:** The Division of Materials Science is engaged in broad areas at Catalysis and Surface Science, Engineering Ceramics and Refractories and Production Technology. It operates laboratories at Parkville, Fitzroy and Fisherman's Bend in Victoria and at Woodville in South Australia. The Production Technology Laboratory, Adelaide has a total staff of approximately 50 people and is a major component of the Division's programs. There are good metallurgical support and computing facilities available. Pilot plant facilities are being established for research into the production of metallic components by a variety of techniques including casting, welding, forging and machining. All research staff are encouraged to sit on liaison committees and to maintain close contacts with manufacturing industry around Australia.

**Position No. 1—Ref. No. 370/350,  
Postdoctoral Research Fellow  
(Materials Science)**

**Duties:** To initiate and develop research projects concerned with the control of the structure of surface coatings applied for protection against wear or corrosion. Experimental equipment available for the projects is an R.F.-D.C. sputter-etch coating unit, a plasma spray unit and various vapour coating units. Current research interests extend also to the morphology and mechanisms of degradation of zinc-rich paints for corrosion control.

**Qualifications:** PhD degree or equivalent in Materials Science. Experience in the structure and properties of coatings applied to metallic components or cutting tools.

**Salary:** Within the range of: Research Scientist \$15,422—\$18,904 or Senior Research Scientist \$19,572—\$22,405 pa.

**Tenure:** This appointment is for a fixed term of 5 years. Superannuation is available.

**Position No. 2—Ref. No. 370/349,  
Production Engineer  
(Metal Forming)**

**Duties:** Leader of an interdisciplinary team concerned with metal forming, particularly with forging of ferrous components. Current emphasis of the research centres on experimental and theoretical analysis of billet deformation and die design. The objective is to reduce production costs in forging by simplifying the production process and reducing the extent of finishing required on forged components.

**Qualifications:** An experienced mechanical or manufacturing engineer is required with PhD qualifications or equivalent, and preferably with a background of mission-oriented research of relevance to manufacturing industry. The appointee should have mathematical ability and experience in computer modelling of deformation processes.

**Salary:** Within the range of: Senior Research Scientist A\$19,572—A\$22,405 pa. Principal Research Scientist A\$23,247—A\$26,368 pa.

**Tenure:** Indefinite appointment with superannuation.

Applications in duplicate, stating full personal and professional details, the names and addresses of at least two professional referees, and quoting the appropriate reference number(s) should reach: The Personnel Officer, Australian Scientific Liaison Office, Canberra House, Maltravers Street, London WC2R 3EH by 21st July, 1979.

Applications in U.S.A. and Canada should be sent to: The Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue N.W., Washington D.C. 20036.

2149(A)

PHLS Centre for Applied Microbiology and Research, Porton, Salisbury

A vacancy exists for a

## Senior Grade Microbiologist

to co-ordinate the scientific work of the Animal House in the Centre. He/she will be responsible to the veterinary surgeon in charge of animal facilities. The animal complex is large, and its functions include the supply of laboratory animals for the research programme of the Centre and the maintenance of primates and other animals experimentally infected with a variety of pathogenic micro-organisms.

Applicants must hold a science degree and have at least four years' postgraduate experience. In addition a qualification in animal technology, e.g. F.I.A.T. is desirable. Applicants should also have considerable experience in the scientific work in an animal house and in particular the containment of pathogens.

Salary: Senior Grade Microbiologist £5,451 to £6,837. N.H.S. terms and conditions of service.

Further details of the post can be obtained from Dr A. Baskerville (Tel. 0980-610391).

Applications stating date of birth, qualifications, experience and publications and naming three referees should be sent to Mrs M. Bushby, C.A.M.R., Porton Down, Salisbury, Wilts. SP4 0JG. 2137(A)

**PHLS**

Public Health Laboratory Service Board.

Cambridge Area Health Authority (T)

### Addenbrooke's Hospital Cambridge

Department of Clinical Biochemistry,  
Cambridge University

## Basic Grade Biochemist

Applications are invited for the above N.H.S. post. Applicants should have a good honours degree in Biochemistry, Chemistry or the equivalent. The possession of some post graduate experience of clinical biochemistry or a Ph.D. in a relevant field will be an advantage.

The department is a joint University/NHS laboratory and encourages research for which there are excellent facilities. Research interests include diabetes, immunoassay, biochemistry of membranes and tissue-specific proteins. The laboratory provides diagnostic services for all the Cambridge hospitals and acts as a regional centre including the East Anglian Regional Hormone Assay Service.

The appointment will provide further training and experience in clinical biochemistry and the opportunity to carry out research. Study for higher qualifications will be encouraged.

Whitley Council conditions apply. Salary scale (under review): Probationary £3,486 (min.); Post Probationary £3,798 (min.) to £4,899 (depending on experience). N.H.S. superannuation.

Application forms and further particulars may be obtained from Professor C. N. Hales, Department of Clinical Biochemistry, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QR. Closing date 27th July. 2074(A)

## THE HANNAH RESEARCH INSTITUTE

FOR STUDIES RELATING TO THE PRODUCTION  
AND UTILISATION OF MILK

### HEAD OF THE BIOCHEMISTRY DEPARTMENT

Applications are invited for the above post. Applicants should have a considerable research experience in animal or microbial biochemistry supported by publications in scientific journals. Preference may be given to persons with a specialist interest in the energy metabolism of the mammary gland.

The post will carry responsibility for the co-ordination and direction of current research into the lipid metabolism of the tissues of ruminant the degradation in the rumen of the structural constituents of herbage and the regulation of microbial metabolism in the rumen, and for the initiation of new work.

Appointment will be in the Senior Principal Scientific Officer grade salary range £11,449 to £12,882 (from August 1, 1979). A non-contributory pension scheme is operating.

Further particulars may be obtained from The Secretary, Hannah Research Institute, Ayr KA6 5HL, to whom applications, giving a curriculum vitae, list of publications and the names of three referees should be forwarded by July 20, 1979. Ref. HRI 35. 2161(A)

## UNIVERSITY OF EAST ANGLIA

Norwich

SCHOOL OF CHEMICAL SCIENCES

ELECTRON ENERGY LOSS

SPECTROSCOPY

OF ADSORBED MOLECULES

Applications are invited for an

S.R.C. POSTDOCTORAL

RESEARCH APPOINTMENT

to work with this new highly sensitive technique of vibrational spectroscopy in order to study the structure of chemisorbed species on single-crystal planes of metals. Such measurements will be made in conjunction with a range of other surface analytical techniques. Experience in the application of electron spectroscopic techniques in surface chemical physics would be of advantage.

The project is a joint one between East Anglia and Queen Mary College, London (Professor J. Pritchard) but the successful applicant will be based at Norwich. The post is tenable for a period close to 3 years depending on the initial salary which will be near point 3 of the RA1A scale (£4,776).

Applicants should write to Professor N. Sheppard, FRSC, or Dr M. A. Chesters, School of Chemical Sciences, University of East Anglia, Norwich NR4 7TJ, enclosing a curriculum vitae and the names and addresses of two referees. 2155(A)

## ROTHAMSTED

EXPERIMENTAL STATION

Harpden, Herts. AL5 2JQ

Applications are invited from suitably qualified scientists for the post of

HEAD

OF THE SOIL MICROBIOLOGY

DEPARTMENT

to succeed Dr P. S. Nutman, FRSC, who retires in Autumn 1979.

The Soil Microbiology Department has over 20 scientific staff and receives many visitors. The scientist appointed will be expected to lead actively the study of soil micro-organisms of agricultural significance and to develop this research in collaboration with other relevant disciplines at Rothamsted.

Appointment in the grade of S.P.S.O., £11,449 by 2 increments to a maximum of £12,882. Non-contributory superannuation.

Apply in writing to the Secretary giving names and addresses of two referees and quoting Ref. 378 by July 20, 1979. Further details on request. 2163(A)

## UNIVERSITY OF LONDON INSTITUTE OF NEUROLOGICAL GRADUATE

RESEARCH ASSISTANT

required to work with a research in a study of effects of neurochemicals on metabolic properties of the axon. Histochemical, biochem and autoradiographic methods will principally be used. Post is suitable recent honours graduate (1 or 2) zoology, physiology or similar subject and the work could be used to gaining a higher degree.

The post is supported by a year grant from Action Research the Crippled Child at a starting of £4,218 (including London allowance).

Applications with curriculum and names of two referees to Prof J. B. Cavanagh, Institute of Neurology, the National Hospital, Queen's Square, London WC1N 3BG. 2085

## ROYAL POSTGRADUATE MEDICAL SCHOOL

University of London

DEPARTMENT OF

CLINICAL PHARMACOLOGY

JUNIOR TECHNICIAN

required to join a team working on isolation and characterisation of different forms of cytochrome from human tissues. The appointed will be trained to enzyme kinetics using a variety of chemical and biochemical techniques including GLC and HPLC. The is supported by a three-year grant from the Medical Research Council.

The successful candidate must possess an O.N.C. or equivalent appropriate subject.

Salary up to £3,138 a year.

Application forms and further particulars may be obtained from Personnel Office, Royal Postgraduate Medical School, 150 Du Cane Road, London W12 0HS, quoting reference number 20/MRC. 2083

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**AUSTRALIAN NATIONAL  
UNIVERSITY  
RESEARCH SCHOOL OF  
CHEMISTRY  
RESEARCH FELLOWS**

Applications are invited for appointment as Postdoctoral Research Fellow in the Research School of Chemistry. Appointments will be for two or three years and may be made in any area of chemistry. Current major research interests in the School are—

**Organic Chemistry:** (Professor A. J. Birch, Mr R. W. Rickards, Dr J. K. MacLeod, Dr L. N. Mander): including organic chemistry of biologically active compounds; synthetic organic chemistry; organometallic chemistry in organic synthesis; metal-ligand reduction studies.

**Inorganic Chemistry:** (Professor G. Hyde, Professor A. M. Sargeant, Dr M. A. Bennett, Dr S. B. Wild (to arrive)): including solid state inorganic chemistry; synthesis, structure and reaction mechanisms of transition metal complexes; biometallic chemistry; organo-transition metal chemistry and chiral arsesines and phosphines.

**Physical and Theoretical Chemistry:** (Professor D. P. Craig, Dr J. Ferguson, Dr R. Bramley, Dr T. R. Elberry): including molecular and crystal theory; spectroscopy and photochemistry; photophysics; disordered materials.

**Theoretical Organic Chemistry:** Dr Radon.

**Mass Spectrometry:** Dr J. K. MacLeod: organic and biological applications; ICR spectrometry.

**X-ray Crystallography:** Dr G. B. Robertson.

**Analytical Chemistry:** Miss B. J. Svenson.

Appointments will be made primarily within these research groups, but proposals for independent research in areas which complement existing programmes will also be considered.

The School is non-departmental and is well equipped to contemporary standards.

Salary on appointment will be in accordance with qualifications and experience within the range \$A15,786–\$A20,606 per annum. Current exchange rates \$A1 : UK£0.53p : S1.10.

Reasonable travel expenses are paid and assistance with housing is given to an appointee from outside Canberra. Superannuation benefits are available.

The University reserves the right to make an appointment or to make an appointment by invitation at any time.

Further information obtainable from the Association of Commonwealth Universities (Apsus), 36

London Square, London WC1H 0PF. There is no application form.

Applicants should supply to the Registrar of the University, P.O. Box Canberra, A.C.T., Australia, by August 31, 1979 a curriculum vitae, of publications and statement of research interests, together with two

port-sized photographs, the names and addresses of three academic referees and the probable date on which a Postdoctoral Research Fellowship, if awarded, could be taken up.

2136(A)

**ANTHONY NOLAN  
BONE MARROW  
RESEARCH LABORATORIES  
St. Mary Abbots Hospital  
Harlow Road, London W8  
SENIOR GRADE PERSON**

needed to take technical and partial administrative charge of these laboratories mainly responsible for tissue culture bone marrow volunteer donors. Applicant should be graduate or senior scientist and have higher qualifications. Considerable experience in tissue culture essential. Apply in writing to Dr C.O. James, Blood Transfusion Westminster Hospital, Dean Ryle, London SW1. 2110(A)

**AGRICULTURAL  
RESEARCH COUNCIL  
INSTITUTE OF  
ANIMAL PHYSIOLOGY  
Babraham, Cambridge CB2 4AT**

Applications are invited from

**GRADUATES**

in Biochemistry or Cell Biology to join a small research group interested in the regulation of ovarian function in mammals. The officer will be involved in a wide range of biological experiments including tissue culture, membrane function, radioimmunoassay of steroid hormones and characterisation of proteins.

Salary in Scientific Officer scale, at present £2,839 to £4,415 per annum, but will be subject to the 1979 Civil Service pay review according to qualifications and experience. Applicants should have, or be expected to graduate with, a pass degree, H.N.C. or equivalent in the relevant biological science. Non-contributory superannuation scheme.

Application form and further details may be obtained from the Secretary of the Institute, quote reference AR521. Closing date: June 28, 1979. 2166(A)

**PAPANICOLAOU  
CANCER RESEARCH  
INSTITUTE**

Applications are invited for

**POSTDOCTORAL**

**RESEARCH ASSOCIATES**

to work with Dr F. Ahmad on (a) isolation, characterisation and control properties of enzymes involved in fatty acid metabolism in bacteria and (b) to work on a neurochemical project involving identification and eventual characterisation of a number of enzymes considered to be present in central nervous system myelin, e.g., protein kinase(s) and phosphoprotein phosphatase(s).

Applications, which should include a curriculum vitae and the names and addresses of three referees, should be sent by August 1, 1979, to Dr F. Ahmad, P.O. Box 016188, Miami, Florida, 33101, USA, from whom further particulars may be obtained. We are an equal opportunity employer. W170(A)

**QUEEN ELIZABETH  
COLLEGE  
Kensington**

(University of London)

**RESEARCH**

**DEMONSTRATORSHIP IN  
PLANT PATHOLOGY**

Applications are invited for a College Research Demonstratorship, available for a 3-year period in the Department of Biology, from October 1979. The Demonstrator will be required to register for a higher degree of the University of London and to conduct research on physiological/biochemical aspects of the resistance of carrot root tissue to *Botrytis cinerea* under the supervision of Dr J. B. Heale.

The Demonstrator will receive a maintenance award of £2,250 per annum if living away from home and £1,370 per annum if living at home plus paid demonstrating (up to 150 hours per year). All fees are also met.

Applicants should have, or expect to obtain a minimum of an upper second class Honours degree in Biochemistry, Botany, Microbiology or related subject, and should write to Dr J. B. Heale (from whom further particulars are available), Biology Department, Queen Elizabeth College, Campden Hill, London W8 7AH, enclosing a curriculum vitae and the names of 2 academic referees. Closing date: June 25, 1979. 2088(A)

**CSIRO AUSTRALIA**  
**Chief of Division**  
**Institute of Physical Sciences**  
**Division of Applied Physics**  
**Sydney, NSW**

Applications are invited for the position of Chief, Division of Applied Physics, from scientists who have an established record of personal research achievement and leadership in an appropriate field of science.

CSIRO is Australia's largest and most comprehensive research organization, having approximately 7,000 employees of whom 2,400 are research and professional scientists. Its broad charter covers research into problems of primary and secondary industries and also such fields of community interest as human nutrition, the environment and the development and use of natural resources. The Organization has recently grouped its research activities in five Institutes and the Division of Applied Physics is one of the Divisions within the Institute of Physical Sciences. Other Divisions in the Institute are Atmospheric Physics, Chemical Physics, Cloud Physics, Computing Research, Environmental Mechanics, Materials Science, Mathematics and Statistics, Radiophysics and the Australian Numerical Meteorology Research Centre.

The Division of Applied Physics is located at Lindfield, a suburb of Sydney, in the new National Measurement Laboratory which was opened in February 1979. In addition there are branch laboratories in Adelaide and Melbourne.

The position of Chief will become vacant upon the retirement of the present incumbent in August 1979. The total staff of the Division is 410 which includes some 160 professional scientists.

The objectives of the Division are:

To establish and maintain the Australian legal standards of measurement as required by the Weights and Measures (National Standards) Act, together with the standards for basic and other physical quantities of importance to Australia.

To promote, together with other national organizations such as National Association of Testing Authorities, and Standards Association of Australia, the development of calibration and other arrangements to enable the community to base its measurements on these standards.

To undertake applied research of importance to industry and the community, utilizing the skills and resources of the Division and to collaborate with industry in exploiting promising developments.

To undertake appropriate physical research utilizing the skills and resources available in the Division.

To undertake any scientific and industrial research that the Executive sees as appropriate for the Division.

To give advice and assistance to industry and the community in areas where the Division has special competence and to provide courses of training where appropriate.

To participate in international scientific and technological activities, especially those arising from Australia's obligations under the Metric Treaty.

To support and co-operate with other nations establishing their own standards and measurement facilities.

A Chief of a Division is responsible for the leadership, development, scientific direction and integration of the research programs of the Division, and is encouraged to promote active collaboration with other Divisions, industry and research bodies working in associated areas. A recent review of the work of the Division of Applied Physics pointed to the need for the Division to improve collaboration with other CSIRO Divisions, industry, and universities. The Chief will be expected to give attention to this aspect of the Division's activities.

The salary for the position is negotiable but will not be less than \$A34,374 pa.

Appointment to the Organization is for an indefinite period and carries Commonwealth Superannuation privileges subject to normal conditions. The position of Chief is offered for a negotiable term of the order of seven years, with subsequent options for a further term, if mutually desired, or for a senior position in the Organization.

Further information on the Division is in a booklet available on request from the Secretary, CSIRO at the address below.

Dr W J McG Tegtart, a member of the Executive of CSIRO, would be pleased to discuss the position with potential applicants. He would also be pleased to receive advice concerning this appointment from people with a particular interest in it. Dr Tegtart can be contacted at the address below.

Applications stating full personal and professional names of at least two professional referees and quoting number 750/762 should reach the Secretary by 14 July, following address: The Secretary, CSIRO, PO Box 225, Dickson ACT 2602, AUSTRALIA.



## VG Scientific Limited

VG Scientific manufactures a range of highly sophisticated electron spectrometers. Due to the success of these products, particularly in the export market, we urgently require more personnel. Vacancies are available for

### Physicists, Engineers and Technicians

with experience in electronics or electron spectrometry. A starting salary in the range £3,500 to £7,000 is envisaged. Usual successful company benefits. Please write or telephone for an application form:

**Mrs S. J. Hudson,**  
**VG Scientific Limited,**  
**The Birches Industrial Estate,**  
**Imberhorne Lane,**  
**East Grinstead,**  
**West Sussex.**  
**Telephone East Grinstead (0342) 25011**

2167(A)

## DIRECTOR OF THE ARC

**Meat Research Institute**  
**Langford, Bristol BS18 7DY**

The Agricultural Research Council invites applications for the Post of Director of the Meat Research Institute which will become vacant on August 31, 1979 as a result of the resignation of the present Director, Professor J. R. Norris, to take up a post in Industry.

The Institute is concerned with applied research to meet the needs of the consumer and the meat industry. In addition it conducts a substantial programme of more fundamental work in animal physiology, muscle structure and biochemistry, together with aspects of food science including microbiology, biophysics and meat flavour.

Candidates should have qualifications or equivalent experience in one or more of the disciplines encompassed by the above description together with a substantial record of research and proven ability in the conduct and management of research.

The post is graded Deputy Chief Scientific Officer, the initial point on the salary scale being £12,773 rising by two annual increments to £13,631. On August 1, 1979 the scale will be £13,359 rising by two increments to £14,128 and on April 1, 1980 to £13,359 rising by two increments to £14,372. There is a non-contributory superannuation scheme. Closing date for applications is July 10, 1979.

Further particulars and application forms may be obtained from Dr Gareth M. Price, Agricultural Research Council, 160 Great Portland Street, London W1N 6DT. 2082(A)

### UNIVERSITY OF DUNDEE DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a  
**POSTDOCTORAL  
 BIOCHEMIST**

Required to work with Dr Grahame Hardie on a project sponsored by the Medical Research Council. The investigation is concerned with the structure of mammalian fatty acid synthase, an enzyme which has seven active sites on a single polypeptide chain (see FEBS Letters (1978) 94, 33-37). Experience in enzymology and/or protein chemistry will be an advantage. The appointment is for one year initially, renewable for a further two years, with starting salary on Range 1A (up to £4,776, under review). Applications, with curriculum vitae, names of two referees, and an indication of date of availability, should be sent to The Secretary, The University, Dundee DD1 4HN by July 6, 1979. Please quote Reference EST/60/79J. 2102(A)

A POSTDOCTORAL SCIENTIST is required to fill a vacancy in the Radiotherapy Research Department of the Institute of Cancer Research. The work of the Department involves the study of the biological effects of radiation and cytotoxic drugs on mouse tumours, human tumours, and human tumour xenografts, making use of tissue culture techniques to assess *in vitro* cell cloning techniques for the direct testing of chemosensitivity in human tumours and familiarity with tissue culture and pharmacology would be an advantage. The appointment will be for an initial period of 3 years, with a possibility of extension. Salary in range £3,909 to £5,129 per annum plus London Allowance of £502 per annum. Applications in duplicate with the names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place, London SW7 3NU, quoting Ref. 300/G/7. 2090(A)

### IMPERIAL CANCER RESEARCH FUND TECHNICIAN/RESEARCH OFFICER

(1) Required in our new Cytogenetics Laboratory to assist with analysing and identifying the human chromosomes in human/mouse somatic cell hybrids. Experience of human metaphase chromosomes, preparation of spreads and standard banding techniques useful. (Reference ES1).

(2) Required to assist with biochemical and immunological assays on somatic cell hybrids for various enzymes, types of collagen and complement components. Experience in a range of biochemical and immunological techniques essential. (Reference ES2).

H.N.C./Degree essential. Salary range £3,615 to £5,256. For further information and application form write or telephone, quoting above reference, to Miss Maddex, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2, on 01-242 0200, ext. 305. 2129(A)

### ULSTER: THE NEW UNIVERSITY SCHOOL OF BIOLOGICAL AND ENVIRONMENTAL STUDIES

**S.R.C. Postgraduate  
 Research Studentship**

Applications are invited from persons who hold or expect to hold a good honours degree in an appropriate subject for a studentship to carry out research in one of the following areas:—

1. Anaplerotic carbon fixation by germinating seeds. (Dr A. M. Flinn.)

2. Gastropod Biology—A range of topics are available including those of pheromonal influences on slug behaviour, the water economy of slugs and its relationship to behaviour and the organisation of the defensive responses of slugs and snails. (Dr T. Cook.)

3. Comparative light and electron microscopic studies of coremium formation in the basidiomycete *Pleurotus cystidiosus* compared with morphologically similar ascomycetous forms. (Dr R. T. Moore.)

Applications, giving an indication of the preferred research topic and the names of two academic referees, should be sent at once to The Registrar, The New University of Ulster, Coleraine, N. Ireland BT52 1SA. Ref: 79/49. 2146(A)

### THE UNIVERSITY OF LEE DEPARTMENT OF PHYSICS

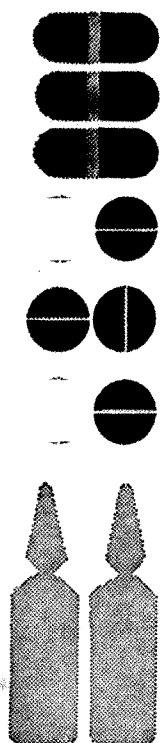
Applications are invited from suitable qualified candidates for a temporary post of

#### RESEARCH FELLOW

in the above Department under the auspices of the I.C.I. Joint Research Scheme. The successful applicant would undertake research, under the direction of Professor I. M. Ward, molecular conformations and orientation in oriented polymers, working on an active research group studying properties and microstructure of polymers. The appointment will be made for a fixed period of three years.

Salary on the 1A scale for Research and Analogous Staff £4,333 to £7,500 (under review), according to qualifications and experience.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS1 9JT, quoting reference number 52/6/79. Closing date for applications: July 1979. 2156(A)



***Head of Regulatory Affairs***  
***five figure salary + car***

This is a new senior appointment, offering international scope, in the Medical & Development Division of Glaxo Group Research Limited at Greenford, Middlesex. The successful candidate will be responsible at Director level for the successful operation of the three Departments concerned with registration, re-registration and control, both in the UK and overseas, of all products developed from laboratory research at Greenford and at Ware, Herts.

We are looking for a candidate, with an academic or industrial background, prepared to inform himself/herself on all aspects of regulatory affairs, and who will represent the Company often with other senior scientists or physicians at meetings with Registration Authorities worldwide. Applications are in-

vited from established physicians or scientists with biological knowledge, who should be at least 35 years of age. They should be capable organisers with the ability to ensure that our submissions to Regulatory Authorities are scientifically correct.

The commencing salary should prove attractive to candidates earning around £10,000 per annum. Other benefits include a non-contributory Pension Scheme, participation in the Group's profitability and generous relocation expenses where appropriate.

Please write, giving details of qualifications and relevant experience, to: Personnel Manager, Glaxo Group Research Limited, Greenford Road, Greenford, Middlesex, quoting reference number /267.

2150(A)

Glaxo Group Research Ltd.

UNIVERSITY OF OXFORD  
DEPARTMENT OF  
GEOLOGY AND MINERALOGY  
RESEARCH ASSISTANT  
IN PALAEOZOOLOGY

**Research Assistant** at either Pre-Postdoctoral level is required for period of up to two years to work under the direction of Dr W. J. J. nedy on the evolution and biogeography of European late Cretaceous ammonite faunas. Candidates should either have, or expect to gain, an Honours Degree (in the case of Predoctoral candidates) or have held their doctorates (in the case of Postdoctoral candidates). Starting salary will be in the range £3,689 to £7,100, depending on age and experience, plus U.S.S. benefits.

applications together with the  
es of two referees, should be  
mitted not later than **June 23,**  
to Dr W. J. Kennedy, Depart-  
s of Geology and Mineralogy,  
s Road, Oxford OX1 3PR, from  
m further particulars may be  
ined. 2130(A)

KENYATTA  
UNIVERSITY COLLEGE  
KENYA

Applications are invited for the following posts in the DEPARTMENT OF CHEMISTRY:-

**1. LECTURER IN PHYSICAL CHEMISTRY.** Applicants must have a good first degree in Chemistry and a Ph.D. in some branch of Physical Chemistry. University teaching experience at the undergraduate and post-graduate levels, and experience in Teacher Education will be considered advantageous. The appointee will be expected to teach Physical Chemistry to undergraduate and M.Sc. students, and to carry out research in some branch of Physical Chemistry.

**2. LECTURER IN INORGANIC CHEMISTRY.** Applicants should hold a good basic degree in Chemistry together with a Ph.D. in Inorganic Chemistry. Experience in Teacher Education would be advantageous. The appointee will be required to teach in the B.Ed. and M.Sc. programmes and carry out research in some field of Inorganic Chemistry.

Salary scales: K£2,016 to 3,312 p.a., (K£1 sterling = £1.28). The British Government may supplement salary in range £4,278 to 4,818 p.a. (sterling) for married appointee or £2,730 to 3,156 p.a. (sterling) for single appointee (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages. Family passages: subsidized housing; S.S.S.F. or F.S.S.U.: non-contributory medical aid scheme. Detailed applications (2 copies) with curriculum vitae and naming 3 referees should be sent by airmail to the Registrar, Kenyatta University College, P.O. Box 43844, Nairobi, Kenya by July 27, 1979. Applicants resident in the UK should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0BD. Further details may be obtained from either address. 2154(A).

**NEWHOUSE, LANARKSHIRE**

# PROGRAMMER

Applications are invited for the above post which has arisen due to expansion of activities in the Automation Unit within the Scientific Development Group of Organon Laboratories Ltd.

The Automation Unit is responsible for providing computing facilities including on-line data capture, statistical analyses and a data-base management system for Chemists and Biologists engaged in research into the development of new drugs. The Automation Unit uses primarily a small network of PDP-11 computers under an RT11/Remote-11 operating system.

The person appointed will assist the Group Leader in the day to day management of these facilities and duties will include writing programmes in FORTRAN. Some experience of scientific programming and PDP-11 computers would therefore be an advantage.

Organon Laboratories Ltd. is located at Newhouse which is situated on the M8 Glasgow-Edinburgh road. Conditions of employment are in keeping with the best modern practice and include salary commensurate with age and experience, Bonus, Sick Pay, Permanent Health Insurance, Pension and Life Assurance Schemes.

Written applications giving details of age, present salary, qualifications and experience to date should be addressed for the attention of the Personnel Manager, Organon Laboratories Ltd., Newhouse, Lanarkshire, ML1 5SH.

2076(A)



UNIVERSITY OF OXFORD  
UNIVERSITY  
LECTURESHIP  
IN FORESTRY

Applications are invited for the post from candidates in the field of aviculture. The salary will be according to age on the scale £4,232 to £6,000 p.a. Details may be obtained from the Administrator, Department of Agriculture, South Parks Road, Oxford OX1 3RB, to whom completed applications (one typed copy) should be sent by 30. 1979. 2078(A)

# Cell Biologist

We are interested in recruiting an innovative post-doctoral research scientist, aged between 25 and 30, to work in our Cell Biology Department. An interest in cell culture techniques in general and their application to the fields of inflammation and rheumatic diseases is desirable. The successful appointee will form part of a multi-disciplinary project group and will be expected to interact with members of the Chemistry, Pharmacology and Biochemistry Departments. He/she will have day to day responsibility for three graduates and technical staff working in our modern laboratories in Welwyn Garden City.

Roche Products Limited is part of a major international pharmaceutical Company based in Switzerland and is itself one of the leaders in the industry in the United Kingdom. The salary payable for this new post will be commensurate with experience and assistance with relocation expenses may be offered where appropriate.

If you are interested in applying for this post then please write or telephone Welwyn Garden City 28128 quoting reference RH6 and giving brief details of your career to Mr J. G. Bennett, Personnel Manager, Roche Products Limited, PO Box 8 Welwyn Garden City, Herts AL7 3AY.

2151(A)


**ROCHE**

## JOHN INNES INSTITUTE DEPARTMENT OF APPLIED GENETICS Three-year temporary appointment BIOCHEMIST/ MOLECULAR BIOLOGIST

required to join a group studying the organisation and expression of the plant genome. The work will involve the isolation and characterisation of a particular nuclear fraction as well as the characterisation of organelle DNA; although the group's work will continue, this particular aspect is expected to be completed by the end of the three year period. The post offers considerable scope for a person interested in the analysis of DNA by a variety of techniques. Further particulars may be obtained from Professor D. Roy Davies.

Applicants should have first or upper second class honours degree in biochemistry or appropriate subject, with at least two years' experience in a relevant field. Appointment as Higher Scientific Officer (£4,101 to £5,448 per annum) (salary under review). Starting salary depending on experience. Non-contributory superannuation.

Applications, quoting reference AG/39, giving full particulars and the names of two referees, should be sent to the Secretary, John Innes Institute, Colney Lane, Norwich NR4 7UH, not later than July 12, 1979. 2111(A)

## WOLFSON UNIT OF CHEMICAL ENTOMOLOGY University of Southampton DEPARTMENT OF CHEMISTRY Applications are invited for the post of RESEARCH ASSISTANT

in the Wolfson Unit of Chemical Entomology from October 1, 1979. The unit is mainly concerned with the development of new methods for insect control and the major duty of the research assistant will be organic synthesis of biologically active compounds. In addition to custom synthesis a wide range of analytical services are offered to industry. The successful applicant will be expected to collaborate closely with industrial sponsors and ideally will be aged between 21 and 25 with a degree (or equivalent) in Chemistry. An interest in Biology would be helpful, although not essential. Part-time registration for a higher degree will be considered. The salary will be on a scale £3,775 to £4,333.

Applications including two referees should be sent as soon as possible to Professor R. Baker, Department of Chemistry, The University, Highfield, Southampton SO9 5NH. Please quote Ref. N. 2158(A)



## THE WORLD HEALTH ORGANISATION

invites applications for a post of Medical Officer (Chief of Cancer Unit) in the Division of Noncommunicable Diseases, W.H.O. Headquarters, Geneva.

The incumbent of the post will be responsible for the planning, organisation and evaluation of the programme in the field of cancer control and prevention at headquarters level, and its coordination with regional office and the International Agency for Research on Cancer.

Applicants should have a medical degree, with specialisation in an area of oncology. Experience in organising and evaluating cancer control programmes, and clinical experience are essential; statistical and research experience, as well as experience in international health work, are desirable. Excellent knowledge of English or French, with working knowledge of the other language is required.

Interested candidates with the required qualifications should write as soon as possible, enclosing a detailed curriculum vitae and quoting reference VN/CAN, to:

**The World Health Organisation  
Personnel  
CH-1211 Geneva 27**

Only candidates under serious consideration will be contacted.

W171(A)

## UNIVERSITY OF LIVERPOOL DEPARTMENT OF MICROBIOLOGY

Applications are invited for the post of a postdoctoral Research Fellow in the Department of Microbiology.

The research which is under the direction of Professor H. R. Perkins, will be concerned with the structure and biosynthesis of the bacterial envelope in relation to antibiotic action.

The post is financed by the M.R.C. and the initial salary will be within the range £4,232 to £5,591 per annum.

Applications, together with the names of two referees, should be received not later than July 13, 1979 by The Registrar, The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/647/N. 2143(A)

## UNIVERSITY OF PENNSYLVANIA POSTDOCTORAL POSITION to study control of Gene Expression and Steroid Hormone/Chromatin Interactions in Cultured Human and Murine Mammary Tumour Cells

Applicant must have strong biochemistry background. Experience with fractionation techniques, nucleic acid hybridization, steroid hormones, antibody production, and/or tissue culture desirable. Send resume, three letters of recommendation and university record to Dr Fred R. Frankel, 249 Johnson Pavilion, University of Pennsylvania, Phila. Pa. U.S.A. 19104. W168(A)

## ANTWERP UNIVERSITY Belgium

CARDIOVASCULAR INSTITUTE  
Applications are invited for a post  
RESEARCH ASSISTANT

tenable for 1 year (starting 01.07 to investigate the ionic activities heart cells.

Salary range: 557.768 BF to 809. BF.

Candidates should have a good degree in biophysics, physiology, medicine or other related sciences with particular interest in electrical properties of excitable membranes.

Applications giving details of cation, experience and names addresses of two referees should be sent by June 20, 1979 to Personnel Department, Universitaire Instelling Antwerpen, Universiteitsplein 1, Wilrijk, Belgium.

Further details can be obtained from Prof. dr. D. L. Brutsaert, Groenenkerkeaan 171, 2020 Antwerpen, 031/30.59.80. W162(A)

## BIOCHEMIST, CHEMIST & IMMUNOLOGIST

available summer 1979 Kiel/Germ to continue in a project on reproductive immunology working with gen analysis and tissue cultures reported by the German Research Foundation. Post doctoral status preferred not absolutely necessary. Send curriculum vitae, credentials and reference to: Dr. Mettler, Laboratory of Human Reproduction, Dept. Obst. and Gyn. University of Kiel, 2300 Kiel, Holtenauerstr. 4, W. Germany. W164(A)



# **SHEFFIELD SOUTHERN HEALTH DISTRICT (TEACHING)**

DEPARTMENT OF BACTERIOLOGY

## **NON-MEDICAL BACTERIOLOGIST**

(Basic Grade or Probationer)

required to work in the Group Department of Bacteriology situated at the Hallamshire Hospital and The Jessop Hospital for Women.

Applicants should possess a 1st or 2nd class Honours Degree in Biological Sciences and should have spent part, preferably the major part, of their course in Microbiology.

Whitley Council Conditions of Service apply.

Salary Scale £3,486 to £4,275 per annum for new entrants to the Health Service. Visits welcomed, by appointment with Mr J. M. Hindmarch, Principal Non-Medical Bacteriologist, Hallamshire Hospital (Telephone 0742-26484, Ext. 2607).

Applications stating age, qualifications and experience and naming two referees to the:

District Personnel Officer, District Personnel Office, B Floor, Hallamshire Hospital, Glossop Road, Sheffield S10 2JF.

Closing date for application: June 25, 1979.

2123(A)

# **UNIVERSITY OF READING**

## **LECTURESHIP**

at

## **THE NATIONAL COLLEGE OF FOOD TECHNOLOGY**

(SECOND ADVERTISEMENT)

Applications are invited for a Lectureship, tenable at the National College of Food Technology, Weybridge, from persons with qualifications and experience in applied microbiology preferably of particular relevance to the food industry. The successful candidate will be expected to contribute to teaching at undergraduate and post-graduate levels and develop an experimental research programme. Opportunities for research are available in all aspects of food microbiology.

The appointment will be made from a date to be arranged with the successful candidate.

Further information may be obtained from the Registrar (Room 214, Whiteknights House), The University, Whiteknights, Reading RG6 2AH by whom applications should be received not later than August 31, 1979.

Applicants for the post advertised in March/April need not reapply.

2107(A)

# **QUEEN ELIZABETH COLLEGE Kensington**

(University of London)

## **POSTDOCTORAL RESEARCH ASSISTANT PHYSICS DEPARTMENT**

Applications are invited for a postdoctoral Research Assistant to work on the development of a set of computer programmes which will produce in real time the neutron diffraction data collected by the D 9 actometer multidetector at Institut e-Langevin, Grenoble. Applicants should ideally have some experience in crystallography and in high and level language computer programming. The work will be carried out mainly at Q.E.C., but will involve regular visits to I.L.L.

The appointment will be for one year from September 1, 1979 in the instance, with the possibility of extension. Initial salary in the range £12 to £15,048 per annum (under 30) plus £502 per annum London allowance.

Applications to Dr C. Wilkinson, Physics Department, Queen Elizabeth College, Campden Hill Road, London W8 7AH from whom application forms are obtainable.

2131(A)

# **UNIVERSITY OF LIVERPOOL DEPARTMENT OF BIOCHEMISTRY**

Applications are invited from biochemists for the postdoctoral post of

## **SENIOR RESEARCH ASSISTANT**

to work on the biochemistry of fluorescent iron-binding chromophores synthesized by some bacteria. A role in iron metabolism has only recently been ascribed to some of these pigments. Familiarity with the concepts and techniques of microbiology would be advantageous. The project is financed for a period of three years.

Initial salary in the range £4,232 to £4,776 per annum with U.S.S. benefits.

Applications, together with the names of two referees, should be received not later than July 5, 1979, by The Registrar, The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/644/N.

2142(A)

# **PORTSMOUTH POLYTECHNIC DEPARTMENT OF BIOLOGICAL SCIENCES**

## **RESEARCH ASSOCIATE**

Postdoctoral graduates required to work on a study of invertebrate macrofauna of marine soft sediments. Supervisor: Dr C. H. Thorp.

Salary: £3,510 to £3,609 per annum. Contract is initially for 2 years.

Application forms and further particulars from the Staff Office, Portsmouth Polytechnic, Alexandra House, Museum Road, Portsmouth PO1 2QQ to whom completed applications should be returned by June 28, 1979. Quoting Reference number: B43.

2072(A)

# **EUROPEAN SCIENCE FOUNDATION**

## **EUROPEAN TRAINING PROGRAMME**

in

## **BRAIN AND BEHAVIOUR RESEARCH**

### **TRAINEESHIPS**

Traineeships of three, six or rarely nine months are offered to promising young scientists working in the area of brain and behaviour research in order to broaden their skills and knowledge in a field other than but related to their own. The grants are given to allow them to learn a particular technique abroad for which training is not offered in their own country. The trainee is expected to return to her/his original post upon termination of her/his training so that her/his institute will in turn be able to benefit from newly acquired skills.

All applications have to be accompanied by a letter of recommendation by the sending institute and by a letter of acceptance from the receiving institute. Preference is given to post-docs under the age of 35.

### **TRAVEL GRANTS**

Travel grants are awarded to allow young research workers to visit another laboratory in connection with an on-going research project or to participate in international meetings and symposia. In addition special grants are to be awarded to students to enable them to take part in the yearly meetings of the European Neuroscience Association (ENA) which this year will take place in Rome on September 10-14.

Applications should be accompanied by letters of recommendation from the sending institute and of invitation from the institute to be visited. Applicants should be postgraduate research workers with the exception of applications to attend the yearly ENA meeting. In this particular case preference is given to postgraduate students.

The deadline for completed applications is March 15 and September 15. Further information and application forms may be obtained from:

Dr Stephanie Zobrist  
European Science Foundation  
European Training Programme in  
Brain and Behaviour Research  
1, quai Lezay Marnésia  
F-67000 STRASBOURG  
FRANCE  
Tel: (88) 35 30 63  
Telex: 890440

W158(A)

# **UNIVERSITY OF OXFORD NUFFIELD PROFESSORSHIP OF ANAESTHETICS**

Electors intend to proceed to an election for the Nuffield Professorship of Anaesthetics which falls vacant on July 1, 1980. The stipend of the professor will be £12,084 per annum. Applications (twelve copies), naming referees but without testimonials, should be received not later than July 27, 1979 by the Registrar, University Offices, Wellington Square, Oxford OX1 2JD, from whom further particulars may be obtained.

2080(A)

Public Health Laboratory Service Board  
61 Colindale Avenue, London NW9 5EQ

## Curator

(Consultant Medical Microbiologist  
OR Top grade Microbiologist)  
**National Collection of Type Cultures**  
**Central Public Health Laboratory**  
**Colindale**

Applications are invited from medical and non-medical graduates for the whole-time post of Curator of the National Collection of Type Cultures. The primary function of the NCTC is to hold and supply authenticated bacterial cultures for education, control, assay, taxonomic and other purposes to PHLS Laboratories, Universities, Medical Schools, Hospitals and Industry. Other functions include the provision of an identification service for bacteria of medical relevance that are difficult to classify, research on bacterial taxonomy (classification, nomenclature, identification), and the provision of freeze-drying facilities for other PHLS Laboratories. A higher qualification is essential.

The Laboratory may be visited by arrangement with the Acting Curator, Dr L. R. Hill, National Collection of Type Cultures, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT. Telephone: 01-205 7041.

Salary on NHS Consultant or Top grade Scientific Officer scale; other terms and conditions of service generally as for appointments in the NHS.

Full particulars may be obtained from the Secretary to the PHLS Board, to whom applications should be sent to arrive not later than July 6, 1979, stating date of birth, qualifications, experience and published work, and naming three referees.

2077(A)

**PHLS**

**Public Health Laboratory  
Service Board.**

READVERTISEMENT—  
IMPROVED SALARY

**QUEEN MARY COLLEGE**  
University of London

**FLUID MECHANICS ON  
THE DISTRIBUTED  
ARRAY PROCESSOR**

S.R.C. is funding research here into the Large Eddy Simulation of Turbulence and has awarded a grant for application of the Distributed Array Processor to this work. The D.A.P., a fundamental development in computer architecture, is particularly suited to solving the Navier-Stokes equations for fluid motion. Applications are invited from those with relevant experience of large programmes (D.A.P. uses a variant of FORTRAN); preference will be given to those with a knowledge of fluid mechanics.

Appointment for 27 months in first instance with initial salary up to £7,388 per annum (including London Allowance), review in October 1979.

Further details available from Professor D. C. Leslie, Nuclear Engineering Department, while applications with curriculum vitae, publications and names of two referees, should be sent to The Registrar (N), Queen Mary College, Mile End Road, London E1 4NS. 2135(A)

**MANCHESTER  
POLYTECHNIC  
DEPARTMENT OF  
CHEMISTRY  
LECTURER II  
IN CHEMISTRY**

Applicants should be broadly experienced in the practice of Analytical Chemistry, and a special interest in the analytical aspects of organic chemistry would be particularly appropriate. Applicants should have a keen interest in contributing to the development of research activity in appropriate fields, and teaching and/or industrial experience would also be an advantage.

The successful applicant will contribute to the teaching of Chemistry (particularly Analytical) on H.N.C./H.C., H.N.D./H.D., Grad. R.I.C., B.Sc. and M.Sc. courses in Chemistry, and also to the service teaching of Chemistry to students on courses in Biological Sciences, Polymer Science and Technology, Food Technology, Science Education etc.

Salary scale: £4,101 to £6,558. For further particulars and application form (returnable by June 30, 1979), please send a self-addressed envelope marked "T/491" to the Secretary, Manchester Polytechnic, All Saints, Manchester M15 6BH. 2079(A)



**Join  
the Nuclear  
Research  
Team**

### RESEARCH OFFICER

**OPPORTUNITY FOR CANADIAN CITIZENS  
LIVING ABROAD**

The Materials Science Branch of the Chemistry and Materials Division at Chalk River Nuclear Laboratories is looking for a scientist to join an active group seeking a fundamental understanding of the effect of irradiation on the creep and growth of zirconium alloys in nuclear reactors. The work will include the formation and migration of point defects in very high purity zirconium and their interaction with trace impurities. The successful candidate will also be expected to perform basic research in the field of irradiation effects and to examine the effect on mechanical properties of zirconium alloys.

Candidates should have a Ph.D. degree in physical metallurgy, solid state Physics or related areas of science. In addition, applicants who have an advanced degree with relevant experience will also receive full consideration. Experience with radiation effects studies, tracer diffusion, and techniques for preparing high purity transition metals would be an advantage.

Salary dependent on qualifications and experience. We offer good benefits package and a removal allowance to assist with moving expenses.

Chalk River Nuclear Laboratories is located 190 kilometres north-west of Ottawa on the Ottawa River. Excellent educational facilities and year-round cultural and recreational activities are available in the Deep River-Pembroke area.

Qualified men and women are invited to apply in confidence quoting file 5A to Employment Supervisor, Atomic Energy of Canada Limited, Research Company, Chalk River Nuclear Laboratories, Chalk River, Ontario K0J 1J0. W161(A)



**Atomic Energy  
of Canada Limited**

**L'Énergie Atomique  
du Canada, Limitée**

**MANCHESTER AREA HEALTH AUTHORITY (T)  
CENTRAL DISTRICT**

### RESEARCH TECHNICIAN

For the University of Gastroenterology Research Laboratory, Manchester Royal Infirmary, which is undertaking investigations into Coeliac disease. The work involves tissue culture of the small intestine. Previous experience in biochemical techniques advantageous.

The appointment is for two years in the first instance. Salary will be in the range of £2,991 to £4,899 per annum depending on qualification and experience.

Informal enquiries to Mr R. Lobley. Tel. 061-273 3300, Ext. 179.

Application form and job description from the Deputy Administrator, Manchester Royal Infirmary, Oxford Road, Manchester M13 9WL. Closing date July 2. 2139(A)

**WELSH NATIONAL  
SCHOOL OF MEDICINE  
(University of Wales)  
DEPARTMENT OF  
MEDICAL BIOCHEMISTRY  
RESEARCH OFFICER**

required in the above Department at Heath Park, Cardiff to work on the development and application of assays of human corticotrophin using labelled antibodies. The project is funded for a period of three years and would be suitable for a biochemistry graduate wishing to read for a higher degree. Salary will be £3,689 per annum.

Further particulars and application forms quoting Ref. No. C38/4/28 may be obtained from the Registrar and Secretary, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN. 2145(A)

**ST GEORGE'S HOSPITAL  
MEDICAL SCHOOL  
(University of London)  
RESEARCH ASSISTANT  
IN BIOCHEMISTRY**

Applications are invited from recent or 1979 honours graduates in biochemistry or chemistry to study mechanisms of biosynthesis, intracellular transport and proteolysis of secreted polypeptides. The appointment is for three years and is expected to lead to a Ph.D. degree. Minimum emoluments from October 1, 1979 £4,277 per annum.

Applications including a curriculum vitae and names and addresses of referees, should be sent immediately to Dr B. M. Austen, Department of Peptide Chemistry, National Institute for Medical Research, Mill Hill, London NW7 1AA. 2134(A)

# newscientist Editor

Dr. Bernard Dixon will be leaving New Scientist this Autumn to pursue other science writing interests. Applications are invited for the post of Editor. New Scientist is the world's leading science news weekly, occupying a unique position between professional journals and news magazines. It appeals to readers with widely diverse interests—students, professional scientists, businessmen and those with a general interest in science and technology. The circulation of the magazine has been growing steadily during the 70's to its current level of over 80,000 copies a week.

Applicants who have not held senior positions in science journalism are unlikely to have the experience required.

Applications will be treated in the strictest confidence and should be made in writing with comprehensive career details to Michael Godfrey, Publisher, New Scientist, IPC Magazines Limited, 2625 King's Reach Tower, Stamford Street, London SE1 9LS.

2138(A)

## UNIVERSITY OF DURHAM

DEPARTMENT OF  
CHEMISTRY

### RESEARCH TECHNICIAN GRADE 5

Applications are invited from suitably qualified persons for an R.C. funded post of a Research Technician (Grade 5) in the S.C.A. group. The post arises from a new S.R.C. funded grant proposal on Structure, Bonding and Reactivity of Polymer Surfaces and will be to February 1981 in the first instance from August 1, 1979 or a mutually agreeable date soon after.

The person appointed will be expected to make routine measurements of high energy (E.S.C.A.) photoelectron Spectrometers, prepare samples and organise spare equipment in the E.S.C.A. laboratories under the direction of Professor D. T. Clark. Initial salary £3,474 per annum.

Application forms and further details from:

Personnel Officer,  
University Office,  
Old Shire Hall,  
Durham DH1 3HP

whom completed forms should be returned by June 29, 1979.

2141(A)

## CHELSEA COLLEGE University of London UNIVERSITY OF GHANA TEMPORARY LECTURESHIP IN GEOPHYSICS

An Inter-University Council supported link scheme has existed since 1976 between the Geology Departments of Chelsea College and University of Ghana. The scheme includes the post of Lecturer in Geophysics under the Home Base Scheme and applications for this post are invited.

The appointment is as Lecturer in the Geology Department, Chelsea College, for a period of two and a half years but the first two will be spent on secondment to the University of Ghana. Duties there will include undergraduate teaching of pure and applied geophysics and research in seismology, including the operation of a seismograph station that has been recording since 1976, and aspects of applied geophysics.

Salary scale while at the home base is £4,232 to £8,452 per annum plus £502 London Allowance.

At the University of Ghana the salary scale Cedis 6,420 to Cedis 9,780 (£1=Cedis 5.50 approx.) would be supplemented under the British Expatriate Supplementation Scheme where appropriate. This scheme provides salary supplementation in the range £6,018 to £7,068 married and £3,504 to £4,296 single (under review), plus appointment grant, allowances for family passages, education allowance and children's holiday visit passages, etc.

Further details and application forms from the Personnel Officer, Chelsea College, Friese Greene House, Chelsea Manor Street, London SW3 3TW. Applications to be received by July 3, 1979.

2084(A)

# CSIRO AUSTRALIA Division of Radiophysics Appointment of Group Leader in Solar Radio Astronomy Group Sydney NSW

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees—2,400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

**General:** The Solar Radio Astronomy Group has a total staff of 36, including 10 research scientists. Solar observations are carried out at the CSIRO Solar Radio Observatory at Culgoora near Narrabri, NSW, where 16 of the staff are resident. The principal observing instruments are the 3-frequency radioheliograph (now being converted to 4-frequency operation), the 8-8,000 MHz radio spectrograph the 24-220 MHz spectro-polarimeter (now being extended to 660 MHz) and a high-sensitivity acousto-optic spectrograph.

Interpretation of the observational data and the development of observing instruments are conducted at the Divisional Headquarters at Epping a Sydney suburb.

A major instrumental development now in progress is the conversion of the radioheliograph to a synthesis telescope using digital correlator techniques. Solar research topics include: emission mechanisms of solar radio bursts; the structure of the corona; characteristics of shock waves and particle emissions associated with radio bursts. The Group conducts collaborative studies with other national and international institutes and will be actively participating in the coming Solar Maximum Mission and other Space Laboratory missions.

**Duties:** The appointee will be responsible for the direction of the research activities of the Solar Group and will be expected to plan areas of research into solar radiophysics and maintain close contact with national and international groups engaged in similar fields.

**Qualifications:** PhD or equivalent qualifications with a background in physical science or engineering and a record of personal achievements in research and leadership.

**Tenure:** Appointment to the Organization is for an indefinite period with superannuation. The position of Solar Group Leader will be for a negotiable term of about five years, with options for further terms if mutually desired, or to a senior position in the Group.

**Salary:** Senior Principal Research Scientist A\$27,790—A\$30,530 p.a.

Applications in duplicate, stating full personal and professional details, the names and addresses of at least two professional referees, and quoting reference number 780/621 should reach: The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 30th August, 1979.

Applications in U.S.A. and Canada should be sent to: The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, N.W., Washington, D.C., 20036, U.S.A.

2148(A)

## CENTRAL PUBLIC HEALTH LABORATORY MEDICAL LABORATORY SCIENTIFIC OFFICER

A.I.M.L.S. or H.N.C.  
in Microbiology

required in the Division of Microbiological Reagents and Quality Control to assist in the production development and quality control of diagnostic reagents.

Applications with full details of age, experience and qualifications to the Personnel Officer, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT. Telephone: 01-205 7041.

2075(A)

## UNIVERSITY OF DURHAM DEPARTMENT OF CHEMISTRY

Applications are invited for the post of Senior Demonstrator in Inorganic/Physical Chemistry for one year, from October 1, 1979.

Initial salary in the range £3,775 to £5,199 per annum, on scale 1A or 1B (depending upon qualifications) plus U.S.S.

Applications (three copies) naming three referees should be sent by June 30, 1979, to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained.

2118(A)



## CSIRO AUSTRALIA

### Division of Materials Science Parkville, Victoria

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees—2,400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

**Field:** Catalysis.

**General:** The Division is engaged in a broad area of Surface Science, Materials Research and Production Technology. It operates laboratories at Parkville, Fitzroy and Fishermen's Bend in Victoria and at Woodville in South Australia. The appointees will be located at the Catalysis and Surface Science Laboratory, University of Melbourne, Parkville, Victoria.

**Position No. 1. Ref. No: 370/348**

## Postdoctoral Research Fellow

**Duties:** To participate in a research group studying catalytic processes for fuel synthesis and processing; particularly reactions of cyclic hydrocarbons and heterocyclics in order to develop new catalysts for hydrotreating of petroleum and coal products.

**Tenure:** The Fellowship will be for a period of five years. Superannuation is available.

**Position No. 2. Ref. No: 370/347**

## Research Scientist

**Duties:** To participate in a research group studying catalytic processes for fuel synthesis and processing; in particular to investigate the preparation, characterization and use of dispersed metallic catalysts for hydrocarbon conversions and related processes.

**Tenure:** Indefinite with superannuation.

**Salary:** (for both positions) Research Scientist A\$15,422—

A\$18,904 pa or Senior Research Scientist A\$19,572—A\$22,405 pa.

**Qualifications:** (for both positions) A PhD degree in Chemistry or Chemical Engineering or equivalent.

Applications in duplicate, stating full personal and professional details, the names and addresses of at least two professional referees, and quoting the appropriate reference number(s) should reach: The Personnel Officer, Australian Scientific Liaison Office, Canberra House, Maltravers Street, London WC2R 3EH by 13th July, 1979. Applications in U.S.A. and Canada should be sent to: The Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue N.W., Washington D.C. 20036.

2147(A)

Dundee College of Technology Department of Molecular & Life Sciences

## SENIOR LECTURESHIP/ LECTURESHIP IN BIOLOGICAL SCIENCE

Applicants should have an honours degree and a higher degree in biochemistry, mammalian physiology, or microbiology and extensive research or industrial experience. The person appointed will be required to make a substantial contribution to the teaching of biological sciences in degree, HND and HNC courses; he/she will also be expected to make a major contribution to course development, research, staff development and departmental administration.

## LECTURESHIPS IN CHEMISTRY (TWO POSTS)

Applicants should have an honours degree and a higher degree in chemistry and suitable industrial or research experience. The persons appointed will be expected to make a contribution to the teaching of chemistry in degree, HND and HNC courses; they will also be expected to contribute to course development, research and departmental administration.

**Salary Scales:** (presently under review) Senior Lectureship—£7,155—£7,962 (bar)—£9,042; Lectureship—£4,056—£7,167 (bar)—£7,698.

Initial placing on these scales will depend upon approved prior experience. Further particulars and application forms obtainable from the Administrative Assistant (Establishment), Dundee College of Technology, Bell Street, Dundee, DD1 1HG, to whom completed application forms should be returned by 29th June, 1979.

2103 (A)

## UNIVERSITY OF DURHAM DEPARTMENT OF CHEMISTRY

Applications are invited for the post of Temporary Lecturer in Organic Chemistry for three years from October 1, 1979. It is hoped that applicants will already have some lecturing experience. An interest in some branch of organofluorine chemistry or polymer chemistry (preparative or structure and bonding by means of photoelectron spectroscopy) and a willingness to join existing groups working in these fields would be an advantage.

Initial salary on the range £4,333 to £8,992 per annum, with superannuation.

Applications (three copies) naming three referees should be sent by June 30, to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained.

2120(A)

## SOUTHAMPTON UNIVERSITY WOLFSON CENTRE FOR ELECTROCHEMICAL SCIENCE RESEARCH SCIENTIST

Applications are invited for the position of Research Scientist in the Wolfson Centre for Electrochemical Science, which is a contract research organisation offering a service to industry in all fields of pure and applied electrochemistry and electrochemical engineering.

Applicants should have a Ph.D. in a relevant subject or equivalent industrial experience. The salary will be within a range from £4,000 to £6,000 per annum (under review). Superannuation benefits, assistance towards relocation expenses.

Applicants should send a curriculum vitae together with the names of two referees to Mr D. A. S. Copland, The University, Southampton SO9 5NH, quoting reference 203/R.

2117(A)

## UNIVERSITY OF BRISTOL DEPARTMENT OF BOTANY RESEARCH ASSISTANT to work on

### Root Surface Microorganisms

A N.E.R.C. funded research assistant post is available for 3 years starting September 1979, to investigate the influence of environmental factors on the abundance of root-surface microorganisms and on exudation by roots. Starting salary £3,689 per annum (scale subject to revision on October 1st, 1979). Opportunity to work for a higher degree. Qualifications required: good honours degree in biology, microbiology or a related subject. Further particulars from, and applications to, Dr E. I. Newman, Botany Department, The University, Bristol BS8 1UG. Closing date for applications July 9, 1979.

2098(A)

Applications are invited for the position of

### ASSISTANT PROFESSOR

(Tenure Track) of engineering science. Positions are available in September 1979 and January 1980. Applicants should possess a Ph.D. in engineering or a related physical science and have demonstrated the ability to generate original research. The positions involve research, teaching graduate and undergraduate courses and the guiding of graduate student research. Applicants should submit a curriculum vitae, the names and addresses of three referees and a statement of teaching and research interests to A. M. Strauss, Head, Department of Engineering Science, M.L. #112, University of Cincinnati, Cinn. OH 45221. The University is an Equal Opportunity Affirmative Action Employer.

W166(A)

## UNIVERSITY OF WARWICK RESEARCH FELLOW IN PHYSICS/PHYSICAL CHEMISTRY

(Collaborative appointment with the University of Cambridge)

Applications are invited for the above post to work in a joint project with Professor J. M. Thomas (Cambridge) and Dr R. F. Pettifer (Warwick) in a study of the characterisation of catalysts by extended X-ray absorption fine structure (EXAFS).

The successful applicant will be based at Warwick University, but will travel frequently to both Cambridge and the Daresbury Laboratory to work with the new synchrotron radiation source.

Applicants should possess a post-graduate degree in either physics or surface chemistry. A knowledge of EXAFS or catalysts would be an advantage, but not essential. The post is offered for three years starting between October 1, 1979 and February 1, 1980 on the research range 1A scale, £4,232 to £7,145 per annum.

Further particulars and application forms available from the Academic Registrar, University of Warwick, Coventry CV4 7AL, quoting ref. No 44/2A/79, to whom completed applications should be returned as soon as possible.

2122(A)

## HERPETOLOGIST HARVARD UNIVERSITY

Harvard University seeks to make a tenure-track appointment in herpetology of an assistant or associate professor in the Department of Biology, who will serve conjointly as an assistant or associate curator in The Museum of Comparative Zoology. Preference will be given to candidates with (1) a strong research program in herpetology, (2) ability to offer instruction at the undergraduate and graduate levels, and (3) commitment to supervise and enhance a major herpetological collection. The closing date for receipt of applications is October 15, 1979, and the appointment will take effect July 1, 1980. Applicants should send a curriculum vitae, statement of research interests, and names of three references to: Herpetology Search Committee, Office of the Chairman, Department of Biology, Harvard University, Cambridge, Massachusetts 02138. Harvard is an Equal Opportunity/Affirmative Action Employer.

W160(A)

## THE UNIVERSITY OF MANCHESTER RESEARCH ASSOCIATE IN MEDICAL BIOPHYSICS

Applications are invited from engineers or physical scientists who wish to join a small interdisciplinary group working on the important problem of incontinence. The appointee would be expected to take a particular interest in models of the bladder and urethra and to apply this work to the design of artificial drainage systems for the management of incontinence. The appointment is for up to three years. Starting salary range per annum: £4,232 to £5,300 (£4,833 to £5,488 from October 1979). Further information from Professor B. R. Pullan, Department of Medical Biophysics, Stopford Building, The University, Oxford Road, Manchester M13 9PT.

2116(A)

To place your  
advertisement in  
these pages

Phone:  
01-831-6901

# Palynologist

We are looking for a Palynologist to apply palynology to the biostratigraphical discrimination, dating and correlation of samples from geological surveys and exploration wells as part of our central service to exploration areas world-wide.

The salary will be determined according to age and experience. Fringe benefits include a non-contributory pension scheme, 4 weeks' annual leave, relocation expenses and excellent sports and social facilities.

If you have a good honours degree in Geology plus at least one year's post-graduate training/experience in palynology and are interested in a career with BP, then please write giving details of your career, qualifications and experience to:

Mrs. L. Fuller,  
Personnel & Administration,  
BP Research Centre,  
Chertsey Road,  
Sunbury-on-Thames,  
Middlesex TW16 7LN.

**BP Research Centre**  
**Sunbury on Thames**

2164(A)

## Agricultural Research Council INSTITUTE FOR RESEARCH ON ANIMAL DISEASES

# BIOMETRICIAN

An experienced BIOMETRICIAN is required in the Statistics Section of the Institute. The successful applicant will be required to specialise in microbiological problems in infectious disease and to identify areas of research which would benefit from greater statistical involvement. The work will also require collaboration with research scientists and supervision of junior statisticians in the Section which has a remote computer link to the ICL 4/70-72 at Rothamsted Experimental Station.

Candidates should have a Degree, HNC or equivalent in Mathematics/Statistics and will normally be expected to have had at least five years' relevant practical post qualifying experience. Salary in a scale (£4,101 to £5,448), currently under review.

The ARC Pension Scheme is non-contributory.

The Institute is set in pleasant surroundings in a rural area with a staff restaurant and good recreational facilities. Accommodation for a single person can be provided if required.



Application Forms from: Secretary, Institute for Research on Animal Diseases, Compton, Newbury, Berkshire quoting reference number 409.

2133(A)

## STUDENTSHIPS

### PORTSMOUTH POLYTECHNIC DEPARTMENT OF BIOLOGICAL SCIENCES

#### S.R.C. C.A.S.E. Studentships

**Post 1 Title:** The use of tree-rings as a means of dating timbers from historical sites.

Supervisor: Dr F. A. Hibbert.

Co-operating body: Department of Environment, Ancient Monuments Laboratory, London.

**Post 2 Title:** Microbial infestation of Diesel fuel storage delivery systems.

Supervisors: Drs R. A. Eaton and E. B. G. Jones.

Co-operating body: Ministry of Defence, Central Dockyard Laboratory, Portsmouth.

#### S.R.C. Studentship

**Post 3 Title:** Oogenesis in developmentally retarded tadpoles of *Xenopus laevis*.

Supervisor: Dr T. A. J. Reader.

#### S.R.C. Advanced Course Studentships

### M.Sc. COURSE IN THE BIODETERIORATION OF MATERIALS

A number of S.R.C. awards are available for suitably qualified students who wish to apply for the above course.

Starting date for all posts: October 1, 1979.

Applications to include a curriculum vitae and the names of two referees should be sent to the Administrative Assistant, Department of Biological Sciences, Portsmouth Polytechnic, King Henry 1 Street, Portsmouth PO1 2DY from whom further information can be obtained if desired.

Closing date for all applications: June 28, 1979. 2071(F)

### UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL S.R.C. C.A.S.E. STUDENTSHIPS

Three S.R.C. C.A.S.E. awards will be available in the Department of Physiology and Pharmacology in collaboration with I.C.I. (2) and Reckitt and Colman from October 1, 1979. They are for research (leading to a Ph.D.) in the following areas:

1. A physiological and pharmacological study of some of the factors contributing to the development of hypertension using rats with arterial hypertension induced by short-term isolation. Supervisor Dr T. Bennett.
2. Two projects on CNS neurotransmitters and their possible role in mental disease. (a) Effects of 5-hydroxytryptamine (5HT) agonists and antagonists on brain 5HT metabolism and release. Supervisor Dr C. A. Marsden. (b) Interactions between CNS thyrotropin-releasing hormone and biogenic amine release. Supervisors Drs G. W. Bennett and C. A. Marsden.

Applicants, honours graduates or prospective honours graduates in physiology, pharmacology or related biological subjects, should write, as soon as possible, giving a brief curriculum vitae and the names and addresses of two referees, to Professor P. H. Fentem, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH.

For further details about the projects please contact the relevant supervisors: Tel. 0602 700111, ext. 3297 (GWB/CAM) or 3600 (TB). 2092(F)

### U.M.I.S.T. POSTGRADUATE STUDIES IN CORROSION SCIENCE CORROSION AND PROTECTION CENTRE

Applications are invited from candidates possessing or expecting a good degree in science or engineering, or a suitable professional qualification, who wish to undertake postgraduate studies in corrosion science and engineering. The U.M.I.S.T. Corrosion and Protection Centre involves over 100 academic, technical and postgraduate staff and students, and is well equipped with a variety of modern electronoptical and electrochemical techniques.

There are S.R.C./C.A.S.E. studentships or equivalent financial support available for research in a number of areas of corrosion and protection, including:

- (1) Electrochemical studies of paints.
- (2) Electrolytic colouring of aluminium.
- (3) Microbial corrosion.
- (4) High temperature corrosion in low oxygen gases.
- (5) Breakdown of oxide scales by sulphur.
- (6) Glasses as anti-corrosive agents.
- (7) Oxidation of steels in Co/Co<sub>2</sub>.
- (8) Oxidation of ion implanted alloys.
- (9) Aluminium anodes for cathodic protection.
- (10) Mechanisms of inhibition of aluminium by paints.
- (11) Corrosion of ion-implanted magnesium.

Research students are expected to register for the degree of M.Sc. (by research) or Ph.D.

Applications will also be considered for the postgraduate courses in Corrosion Science or Terotechnology offered by the Centre.

All applications and enquiries should be addressed to Professor G. C. Wood, Corrosion and Protection Centre, University of Manchester Institute of Science and Technology, P.O. Box 88, Manchester. 2126(F)

### ROYAL HOLLOWAY COLLEGE (University of London) S.R.C. STUDENTSHIP DEPARTMENT OF BIOCHEMISTRY

Applicants should have, or expect to gain this year, a good honours degree in Biochemistry for this research studentship tenable for 3 years and leading for a Ph.D. degree. The project, under the direction of Dr C. Rider, is an investigation of multiple molecular forms of mammalian enzymes of citrate metabolism and a variety of techniques in enzymology will be employed.

Applications together with names and addresses of two referees should be sent to the Registrar, (N) Royal Holloway College, Egham Hill, Egham, Surrey. 2086(F)

### UNIVERSITY OF DURHAM DEPARTMENT OF PHYSICS C.A.S.E. STUDENTSHIP

for work on readout noise sources (involving slice level testing), in SILICON DIODE ARRAYS in collaboration with I.P.L. Dorchester. The main interest is in low light level imaging for astronomy.

Applications should be sent to Dr J. M. Breare, Department of Physics, Science Laboratories, South Road, Durham DH1 3LE (Tel: 0385 64971), from whom further particulars may be obtained. 2091(F)

UNIVERSITY OF  
LIVERPOOL  
DEPARTMENT OF  
METALLURGY AND  
MATERIALS SCIENCE  
S.R.C. C.A.S.E.

AND A.E.R.E. HARWELL

sponsored Research Studentships

Applications are invited from good honours (1st or 2(1)) graduates in Metallurgy / Materials Science or related Physical Sciences for three year sponsored research studentships tenable from October 1, 1979. The research projects are in the following areas:

- (a) Structure and Embrittlement of Low Alloy Ferritic Steels (two positions).

Research into the relation between micro-structure, segregation of alloy and impurity elements to grain boundaries and susceptibility to intergranular fracture in ferritic steels used for power engineering structures in collaboration with A.E.R.E. Harwell. Supervisors: Prof. B. L. Eyre and Dr B. C. Edwards.

- (b) Stress Corrosion Cracking in Aluminium Alloys.

Research into the relation between the atomic structure of grain boundaries and observed rates of intergranular stress corrosion in commercial aluminium alloys in collaboration with Alcan Industries. Supervisors: Dr R. C. Pond and Dr C. Tuck.

- (c) Light-Ion Bombardment of Non-metallic Materials.

To investigate the effects of light, gas ion bombardment on the micro-structure and surface of ceramic materials having potential for use in fusion reactors in collaboration with the Culham Laboratory. Supervisors: Dr D. J. Bacon and Dr K. Ereus.

- (d) Studentships and projects are also available in other areas, viz. heat treatment of steels, computer simulation of crystal defects, development of composite materials, mechanical properties of metals, properties of cellular materials and the properties of bio-materials.

Applications should be received as soon as possible by the Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote ref. RV/630/N. 2105(F)

UNIVERSITY OF  
LIVERPOOL

DEPARTMENTS OF BOTANY  
AND INDUSTRIAL STUDIES

Applications are invited for a S.R.C./S.S.R.C. Research Studentship tenable from October 1979 to study the importance of zinc and copper chelation in the tolerance of plants to these metals and the occurrence of chelates in polluted environments. The project will be jointly supervised by the Departments of Botany and Industrial Studies.

Applicants must possess or expect to gain a good honours degree in chemistry or a biological science. Applications, including details of academic experience and the names of two referees, should be received not later than July 2, 1979, by The Registrar, The University, PO Box 147, Liverpool L69 3BX. Quote Ref: RV/641/N. 2127(F)

N.E.R.C. RESEARCH  
STUDENTSHIP

Applications are invited for a Ph.D. studentship in Fish Physiology. The title of the project is "The effects of exercise, diet and pollutants on protein metabolism in fish." The successful applicant would be expected to possess a 1st or a 2 (1) Honours degree or the equivalent in Zoology, Biology, Physiology or Biochemistry. Applications, which should include the names of two referees, should reach Professor G. Goldspink, Department of Zoology, University of Hull, not later than July 7, 1979. 2140(F)

UNIVERSITY OF  
LIVERPOOL  
DEPARTMENT OF  
MICROBIOLOGY

RESEARCH STUDENTSHIPS

1. S.R.C. (C.A.S.E.) Award to Dr G. O. Humphreys in association with Dr P. Barth, Corporate Laboratories, I.C.I. Ltd., entitled "Heterospecific expression problems in DNA cloning" to study the barriers to heterospecific expression by using hybrid plasmids. It is proposed to seek plasmids occurring naturally in *Arthrobacter* and to prepare hybrid replicons between these and others from *E. coli*.

2. S.R.C. Studentship. The project, to be undertaken with Dr C. Edwards, is entitled "Bioenergetics of the microbial cell cycle". Synchronous cultures of *Azotobacter vinelandii* grown under different conditions will be used to examine the periodic changes in respiratory events such as  $QO_2$  effects of inhibitors and ATPase levels.

3. M.R.C. Studentship. The project, to be undertaken with Professor H. R. Perkins, is entitled "Peptidoglycan synthesis by *Neisseria gonorrhoeae*". The type of peptidoglycan synthesized in an *in vitro* system will be studied in relation to the action of various  $\beta$ -lactam antibiotics, which have different effects.

All studentships commence October 1, 1979. Applicants should hold or expect to obtain at least an upper second class Honours degree in Microbiology, Biochemistry or other relevant discipline.

Applications, together with details of academic experience and the names of two referees, should be received not later than July 13, 1979, by the Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote Ref. RV/646/N. 2144(F)

UNIVERSITY OF SURREY  
DEPARTMENT OF  
BIOCHEMISTRY  
S.R.C. C.A.S.E.

RESEARCH STUDENTSHIPS

Research studentships, for work on the topics listed below, are available in the Department of Biochemistry. The studentships, which are financed by the Science Research Council in collaboration with industry and other organisations, commence on October 1, 1979 and are tenable for three years.

1. Inter-species variations in microsomal oxygenation reactions, in collaboration with The Flour Milling and Baking Research Association, Chorleywood.
2. The nutritional effects of different varieties of bran, in collaboration with The Flour Milling and Baking Research Association, Chorleywood.
3. Effects of the lysosomal stabiliser, carbenoxolone, on carcinogenesis, in collaboration with Biorex Laboratories Limited, London.
4. Stereospecific drug modification using Cytochrome P-450, in collaboration with May and Baker Limited, Dagenham.
5. Development of an inhibitor-based EIA for drug analysis in serum, in collaboration with The Royal Sussex Hospital, Brighton.
6. Metabolism and excretion of monosubstituted imidazoles in rat and dog, in collaboration with Pfizer Limited, Sandwich.
7. Molecular studies of the fidelity of DNA repair, in collaboration with Shell Toxicology Laboratory, Sittingbourne.

Applicants must have, or expect to obtain, first or upper second class honours in Biochemistry or a related subject. Applications, stating the topics of interest and enclosing a curriculum vitae and the names and addresses of two referees should be sent without delay to:

Senior Academic Administrator,  
Department of Biochemistry,  
University of Surrey,  
Guildford,  
Surrey GU2 5XH. 2112(F)

U.M.I.S.T.  
University of Manchester  
Institute of Science  
and Technology  
Ph.D. RESEARCH IN  
FIBRE PHYSICS

Studentships are available for research in the following areas:

1. Micromechanisms involved in the texturing of synthetic fibres (S.R.S./C.A.S.E. Studentship with I.C.I. Fibres).

2. Thermomechanical response of synthetic fibres (S.R.S./C.A.S.E. Studentship with the Cotton Silk and Man-Made Fibres Research Association).

3. Fracture mechanism in oriental polymers: fibres and films (industrially sponsored).

Applicants should hold or expect to obtain a good honours degree in physics, materials science, engineering or a related subject, and should apply as soon as possible to Dr C. P. Buckley, Department of Textile Technology, UMIST, Manchester M60 1QD, from whom further details may be obtained. 2125(F)

UNIVERSITY OF  
NOTTINGHAM  
DEPARTMENT OF BOTANY  
S.R.C. C.A.S.E.

RESEARCH STUDENTSHIP

Applications are invited from persons holding or expecting to receive a First or Upper Second Class Honours Degree in Botany or in any of the Biological Sciences for a C.A.S.E. Studentship in collaboration with Asner Seeds Limited of Leicester to work on the somatic hybridisation of Pelargoniums using protoplasts.

Applications with the names and addresses of two referees and a curriculum vitae should be sent to Dr J. B. Power, Department of Botany, University of Nottingham, Nottingham. 2093(F)

UNIVERSITY OF GLASGOW  
DEPARTMENTS OF PATHOLOGY  
AND BIOCHEMISTRY  
M.R.C.

PARTNERSHIP AWARD

Applications are invited from graduates with an upper second class honours degree for a 3-year research studentship involving the analysis of monoclonal antibodies to human thyroglobulin in normal and diseased tissue. The successful applicant will be expected to work in both the Royal Infirmary and the Biochemistry Department at the University. Applications should be made by July 31 to Dr A. M. Campbell, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ.

In reply please quote Ref. No. 4385M. 2099(F)

UNIVERSITY OF GLASGOW  
DEPARTMENT OF BOTANY  
Applications are invited for an  
S.R.C. C.A.S.E.  
STUDENTSHIP

tenable for three years from October 1979 to work on the resistance of potato tuber tissue to *Phytophthora infestans*. The research will offer interesting and varied opportunities for candidates interested in both the fundamental aspects and practical applications of research into mechanisms of disease resistance. It is expected that three months of each year will be spent with the collaborating institute, the Scottish Plant Breeding Station.

Further details may be obtained from Dr D. D. Clarke, Botany Department, Glasgow University, Glasgow G12 8QQ, to whom applications should be sent, naming two referees, by June 23, 1979. 2101(F)

IMPERIAL COLLEGE  
COST AND ENERGY  
EVALUATION OF  
MINERAL EXTRACTION  
PROCESSES

A Science Research Council C.A.S.E. STUDENTSHIP is offered in association with the Department of Industry, Warren Spring Laboratory, for a study to develop a Methodology of Cost and Energy Evaluation of Mineral Extraction Processes. The methodology will be developed with particular reference to the extraction of alumina from indigenous U.K. resources of aluminiferous materials.

Candidates from any background will be considered, but the study would be of particular interest to those with experience in mineral technology, extraction metallurgy, chemical engineering or chemistry.

Applicants should have, or expect to obtain a 1st or upper 2nd class honours degree. Applicants should send details of their qualifications plus the names and addresses of two referees, to Professor R. N. Pryor, Head of Department, Department of Mineral Resources Engineering, Imperial College of Science and Technology, Prince Consort Road, London SW7 2BP. 2152(F)

LONDON SCHOOL OF  
HYGIENE AND TROPICAL  
MEDICINE  
(University of London)  
Keppel Street, WC1E 7HT  
SCIENCE

RESEARCH COUNCIL:  
C.A.S.E. STUDENTSHIP

Applications are invited from candidates who hold or expect to obtain this summer at least an upper second class honours degree in Biochemistry or another appropriate discipline for a Research Studentship, tenable at the School in conjunction with Glaxo Group Research Ltd for three years from October 1, 1979. The project will be supervised by Dr M. W. Stewart of the Immunology Unit, Department of Medical Biology, and Dr M. W. Elves of Glaxo; and will be concerned with the study of immunological mechanisms involved in antigen-antibody complex disease and the possible modulation of these mechanisms. Applications, consisting of curriculum vitae and naming two referees, should be sent to the Secretary (A1) at the School. 2153(F)

THAMES POLYTECHNIC  
SCHOOL OF  
BIOLOGICAL SCIENCES  
S.R.C. STUDENTSHIP

Applications are invited from holders, or those expected to obtain a good honours degree for a S.R.C. studentship. The project will either: (i) An investigation of the purification of proteins by desorption from mixed-function, non-specific adsorbents using biospecific ligands desorbing agents ("affinity-elution chromatography") and is suitable for biochemist or biologically-oriented chemist. (Supervisor: Dr R. J. Ye or (ii) an investigation into the degradation pathway of the herbicide 2,4-dichlorophenoxy acetic acid *Acinetobacter* sp. and the induction of the enzymes involved, and is suitable for a biochemist or chemist. Some experience of microbiology preferable. (Supervisor: Dr A. R. Smith).

Further particulars and form application may be obtained from Staffing Officer, Thames Polytechnic, Wellington Street, London SE18 6 to whom completed application should be returned by June 26, 1979. 2096(F)



**UNIVERSITY OF ABERDEEN**  
DEPARTMENT OF CHEMISTRY  
S.R.C. C.A.S.E.  
STUDENTSHIPS 1979

Applications are invited for three A.S.E. studentships, two in cooperation with Borax Holdings, Ltd., and one in cooperation with British Gas, work in the undernoted fields.

- (1) Spectroscopic (UV and visible) and electrochemical study of the interaction of sulphide ions with metal ions in molten sodium borate. (Drs. J. A. Duffy and M.D. Ingram)
- (2) The effect of borate fluxes on sintering and solid-melt equilibria in ceramic bodies, and the relation between thermal history, phase composition microstructure, strength and optical properties. Project would also suit applicants with materials science or mineralogical background. (Dr. F.P. Glasser)

For both projects, a period of two months a year will be spent in the Max Research Centre, Chessington, Surrey.

(3) Fundamental reactions relevant to methane combustion. (Dr. L. Bateman)

A period of two months a year will be spent at the London Research Station of the British Gas Corporation. Applicants holding or expecting to obtain a first or upper second class honours degree should communicate with the designated supervisor (Meston Smith, Old Aberdeen AB9 2UE). 2113(F)

**UNIVERSITY OF WARWICK**  
RESEARCH STUDENTSHIPS  
BIOLOGICAL SCIENCES

Research Studentships in Microbiology (1, 2 and 3) or Virology (4) leading to a Ph.D. are available in the Department of Biological Sciences as follows:—

- S.R.C./C.A.S.E.: "Methanotrophs: gums and polysaccharides and taxonomy" in conjunction with British Petroleum, Sunbury-on-Thames, under the supervision of Professor R. Whittenbury.
- S.R.C./C.A.S.E.: "The Production of Methanol by Micro-organisms" in conjunction with British Petroleum, Sunbury-on-Thames, under the supervision of Dr H. Dalton.
- N.E.R.C.: "The significance of methylobacteria in nitrification in the aquatic environment" under the supervision of Dr C. S. Dow.
- M.R.C./PARTNERSHIP: "The study of interspecies recombination in rotaviruses" in conjunction with Dr T. H. Flewett, East Birmingham Hospital, under the supervision of Dr M. A. McCrae.
- Applicants should write direct to Professor R. Whittenbury, Biological Sciences, University of Warwick, Coventry, CV4 7AL, giving a brief curriculum vitae and the names of two academic referees. 2073(F)

**UNIVERSITY OF BRADFORD**  
SCHOOL OF  
MEDICAL SCIENCES  
S.R.C. C.A.S.E.  
STUDENTSHIP

Applications are invited for a C.A.S.E. studentship tenable from October 1979 research on the adherence of oral bacteria related to their cell wall composition and conditions of growth. The project will involve biochemistry, molecular biology and genetics. The work will be carried out in collaboration with the Microbiology Division of the National Institute for Medical Research. Applicants should hold, or expect to obtain a First or Upper Second Class Honours Degree in Microbiology or Biochemistry, and the successful applicant will be required to enter for a higher degree. Further details of the research and application forms are available from Dr H. D. Hoghue, School of Medical Sciences, University of Bradford, BD7 1DP. To complete applications must be received asap. Ref. RS/MS/1/X. 2109(F)

**THE UNIVERSITY OF LEEDS**  
DEPARTMENT OF  
ORGANIC CHEMISTRY  
Research  
Studentships

Applications are invited for the following S.R.C./C.A.S.E. Studentships tenable in the Department of Organic Chemistry from October 1, 1979 in the following areas:

- (1) New  $\beta$ -lactam antibiotics
- (2) New cycloaddition reactions
- (3) Antiviral and antifungal agents

Applicants should hold, or expect to obtain, a First Class or Upper Second Class degree from U.K. universities (or C.N.A.A.) or Grad R.I.C. of equivalent standing. Further information and application forms are available from Professor P. G. Sammes, Department of Organic Chemistry, The University of Leeds, Leeds LS2 9JT. 2124(F)

**UNIVERSITY OF DURHAM**  
DEPARTMENT OF CHEMISTRY  
C.A.S.E. STUDENTSHIPS

Applications are invited for the following C.A.S.E. awards:

1. Spectroscopic Studies of Adsorbed Species and
2. A Neutron Scattering Study of Adsorbed Species: (Dr J. Howard and Professor T. C. Waddington)
3. E.S.C.A. Investigations of the Kinetics and Mechanism of the Weathering of Polymeric Materials and
4. Electron Spectroscopy: Investigations of Technologically Important Surfaces: (Professor D. T. Clark)
5. The Determination of Optical Constants of Liquids and Solutions in the Submillimetre Region: (Dr J. Yarwood)
6. Hydrocarbonylation Reactions for Alcohol Synthesis: (Dr M. Kilner)
7. Syntheses via Fluorinated Anions: (Professor R. D. Chambers).

Several of the above carry payments in addition to the normal S.R.C. rates for research studentships. The Chemistry Department is modern, research orientated, and possesses a very wide variety of physical and chemical techniques which are available to all research workers within the Department.

Applicants should hold, or expect to obtain a first or upper second class honours degree in chemistry (1→7), chemical physics (1→5) or physics (2→5). Further details may be obtained from the staff indicated, by writing to the Chemistry Department, University of Durham, South Road, Durham DH1 3LE. 2094(F)

**UNIVERSITY OF GLASGOW**  
DEPARTMENT OF  
BIOCHEMISTRY  
in cooperation with I.C.I.  
S.R.C. C.A.S.E.  
STUDENTSHIP

An award will be tenable from October 1, 1979 in support of research on the effects of Tamoxifen and other anti-oestrogens on uterine transcription.

Applicants who should possess or expect to obtain a First or Upper Second Class Honours degree in Biochemistry or a related subject should write to Dr J. T. Knowler, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ. 2100(F)

**UWIST**  
(University of Wales)

**S.R.C. C.A.S.E.  
STUDENTSHIP**

Applications are invited for a S.R.C. C.A.S.E. studentship tenable from 10th October 1979 to investigate the response of aquatic micro-organisms to stress which is induced by the presence of pesticides; it is intended to use estimates of bacterial activity to monitor these stresses. This is a collaborative project between Dr. J. C. Fry (UWIST) and Dr. N. J. Poole (ICI Plant Protection Division, Jealott's Hill, Bracknell, Berks.). The investigation which involves both field and laboratory experiments will be carried out at both centres. ICI will supplement the SRC studentship with £200 per annum (normally tax free) and will cover the additional expenses incurred while working at Jealott's Hill.

Applicants, who must possess or expect to obtain a first or upper second class degree, should send a letter of application immediately to Dr. J. C. Fry, Department of Applied Biology, University of Wales Institute of Science and Technology, King Edward VII Avenue, Cardiff, Wales CF1 3NU. Each applicant must provide a curriculum vitae and the names of two referees. 2070(F)



**Plant Protection  
Division**

**UNIVERSITY OF READING**

DEPARTMENT OF AGRICULTURAL BOTANY  
S.R.C. C.A.S.E. STUDENTSHIP

Applications are invited for a Research Studentship tenable from October 1, 1979 to investigate the breeding of raspberry-blackberry hybrids of the Logan type, and especially the use of new sources of spinelessness. The project will include chromosomal and genetic studies. The student will spend some weeks to each year at the Scottish Horticultural Research Institute, Dundee, which is the Co-operating Body, and there will be financial support for these visits.

Candidates should have or expect to obtain a good Honours degree in Agricultural Botany or Botany, or a suitable biological degree. The successful applicant will be registered for a higher degree.

Applications including details of academic experience and the names and addresses of two referees should be sent as soon as possible and not later than June 15 to Dr. J. K. Jones, Department of Agricultural Botany, Plant Science Laboratories, University of Reading, Whiteknights, Reading, RG6 2AS, from whom further information may be obtained. 2108(F)

**PRESTON POLYTECHNIC**  
S.R.C. POSTGRADUATE  
RESEARCH STUDENTSHIPS

Applications are invited for S.R.C. Postgraduate Studentships on one of the following topics:

1. Climatic and biotic factors governing the colonisation of timber joinery by micro-fungi;
2. Photosynthesis in algae and bacteria;
3. Energy metabolism: effects of diet and exercise.

Further details and application forms can be obtained from

The Clerk,  
Biology Division,  
Preston Polytechnic,  
Corporation Street,  
Preston PR1 2TQ

Closing date: June 29, 1979.

2097(F)

**UNIVERSITY OF DURHAM**  
ENGINEERING GEOLOGY  
LABORATORIES  
S.R.C. C.A.S.E.  
RESEARCH STUDENTSHIPS

Applications are invited from engineers or geologists holding or expecting to obtain a good honours degree for a project, starting on October 1, 1979, on the effects of ground movements caused by excavation. The co-operating body is the Northumbrian Water Authority. Further information and enquiries to Dr P. B. Attewell, Engineering Geology Laboratories, University of Durham, South Road, Durham DH1 3LE. (Tel: Durham (0385) 64971, Ext: 411) by June 30, 1979. 2121(F)

## FELLOWSHIPS

## EMBO

European Molecular Biology Organization

LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY  
AUTUMN 1979 AWARDS

Next deadline: August 31, 1979

EMBO long term fellowships are initially awarded for one year. Applications for a renewal for a second year and subsequently in cases of exceptional scientific merit for a third year are considered.

To be eligible a candidate must hold a doctors degree. Preference will be given to European and Israeli applicants wishing to work within Europe or Israel. EMBO long term fellowships are not, however, awarded for exchanges between laboratories within any one country. Applications for fellowships to be held outside Europe and Israel are considered but they have a lower priority, as do applications from non-European scientists wishing to work in Europe or Israel.

Successful applicants will be notified of their awards on October 29, 1979.

Further details and application forms may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, 69 Heidelberg 1, Postfach 1022.40, F. R. Germany. W156(E)

## RESEARCH FELLOWSHIP

A Postdoctoral Cytogeneticist or Molecular Biologist, with experience in human karyotyping, is required to participate in a project on the analysis of chromosomes from human solid tumours.

The appointment is for three years. Salary with entry according to qualifications and experience within range £5,823 to £7,129 inc. L.A. For further information telephone Dr E. Solomons (0865: 511262, ext. 347 until July 1, and then at 01-242 0200, ext. 305). Applications with curriculum vitae and names of two referees should be sent to the Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2, quoting ref. 287/79.

2128(E)

UNIVERSITY OF SURREY  
DEPARTMENT OF MICROBIOLOGY  
POSTDOCTORAL  
RESEARCH FELLOWSHIP

A postdoctoral research fellow is required for a 3-year M.R.C. supported project to determine the DNA sequence of the R46  $\beta$ -lactamase gene. Experience in DNA sequencing or recombinant DNA technology is essential. The starting date will be October 1, 1979.

The salary will be on research grade 1A, starting at £3,883 per annum (under review).

Applications in the form of a curriculum vitae, together with the names and addresses of two referees, should be sent as soon as possible to Dr J. W. Dale, Department of Microbiology, University of Surrey, Guildford, Surrey GU2 5XH. 2089(E)

## ASSISTANTSHIPS

UNIVERSITY OF  
CAMBRIDGE  
DEPARTMENT OF BOTANY  
GRADUATE  
RESEARCH ASSISTANT

Applications are invited for a graduate research assistantship for work on the development and ecological significance of mycorrhiza in seedlings of contrasted types in chalk grassland. The post, which is supported

by the Natural Environment Research Council, is for a period of three years from September 1, 1979.

The salary will be on the scale 1B, starting at present at £3,689 per annum. A suitable candidate would be expected to register for the degree Ph.D.

Applications including curriculum vitae and names and addresses of two referees should be sent as soon as possible to Dr P. J. Grubb, Botany School, Downing Street, Cambridge CB2 3EA, from whom further particulars may be obtained. 2165(P)

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY  
DEPARTMENT OF BIOCHEMISTRY

## POSTGRADUATE RESEARCH ASSISTANTSHIP

A Postgraduate Research Assistant is required to work with an active research group studying pancreatic endocrine function in experimental obesity. The position will be available from mid-July. Previous experience would be welcomed but is not essential.

The starting salary will be within the range £3,718 to £5,333 per annum (according to age and experience) plus £502 London Allowance and U.S.S. benefits.

Applications, including a curriculum vitae and the names of two referees, should be sent, as soon as possible, to Dr Anne Beloff-Chain, Department of Biochemistry, Imperial College, London SW7 2AZ. 2132(A)

## CONFERENCES

RADIATION EMERGENCY ASSISTANCE  
CENTER / TRAINING SITE


announces an

## INTERNATIONAL CONFERENCE

THE MEDICAL BASIS FOR  
RADIATION ACCIDENT PREPAREDNESSOctober 18-20, 1979  
Oak Ridge, Tennessee

For information and registration write:

REAC/TS Conference  
Oak Ridge Associated Universities  
P.O. Box 117 / Oak Ridge, TN 37830

Sponsored by the Department of Energy

As an organization accredited for continuing medical education, Oak Ridge Associated Universities, Oak Ridge, Tennessee 37830 certifies that this continuing medical education activity meets the criteria for 16 credit hours in Category 1 of the Physician's Recognition Award of the American Medical Association.

W169(C)

INTERNATIONAL  
CONFERENCE

on

"The Organisation and Expression  
of the Mitochondrial Genome"

Organisers:

A. M. Kroon, The Netherlands,  
and  
C. Saccone, Italy

In the series of annual meetings in Bari, Italy, a conference on the above subject will be held on June 23-28, 1980. Those interested to attend this meeting, which will deal with various aspects of the biogenesis of mitochondria are requested to contact the conference secretariat:

Istituto di Chimica Biologica,  
Facoltà di Scienze,  
Via Amendola 165/A,  
70126 Bari, Italy.  
Tel: 080-339907.The total number of participants  
will be 100. W157(C)

## CONFERENCE

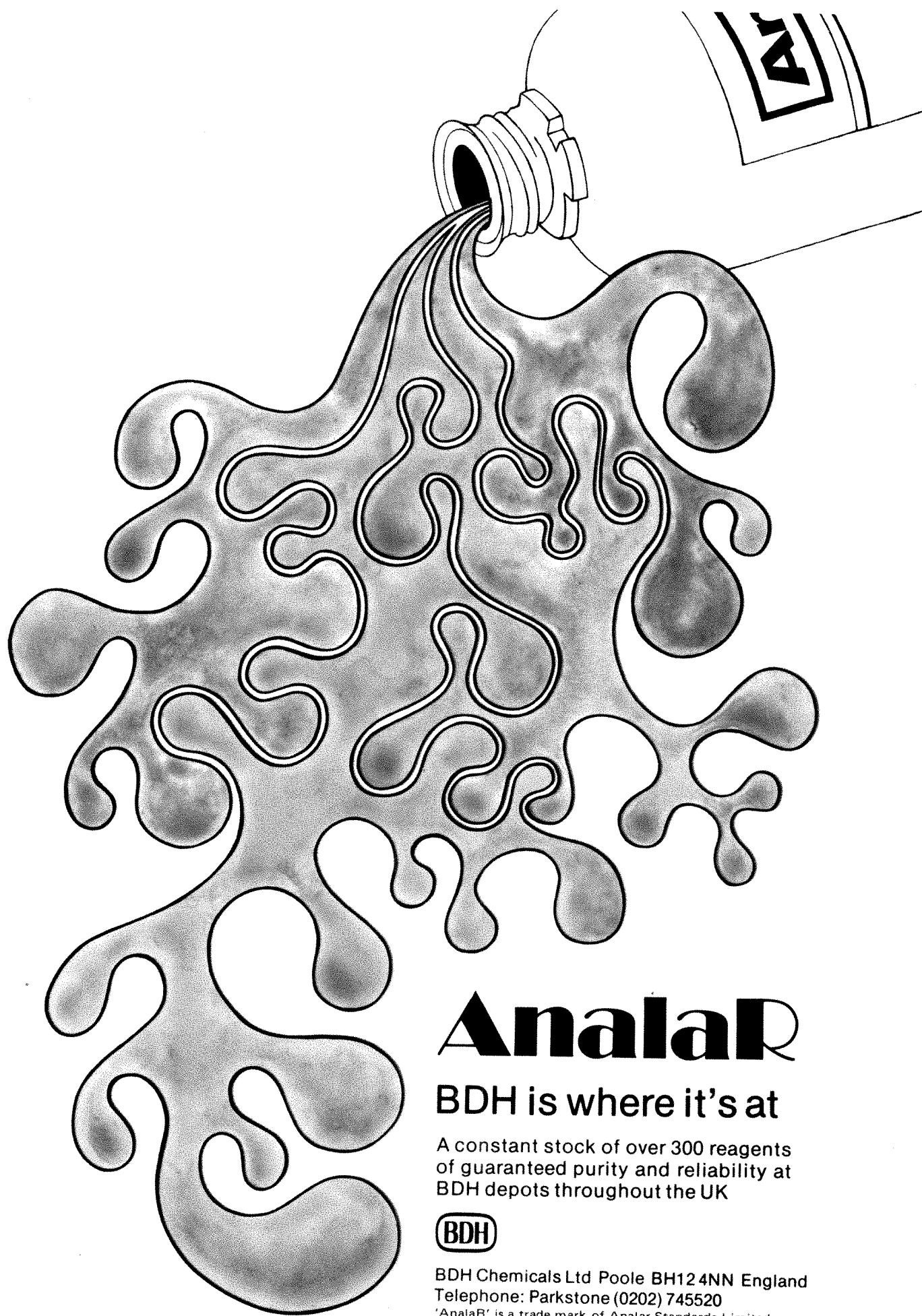
The Sixth Cold Spring Harbor Conference on Cell Proliferation will be held at the Cold Spring Harbor Laboratory from September 4 to 1979. The title of the Conference will be *Viruses in Naturally Occurring Cancers*. For this meeting we plan to bring together leading investigators with diverse backgrounds whose interests vary from molecular biology and genetics to epidemiology.

Topics to be considered include Herpes viruses, papova and adenoviruses, and RNA tumour viruses.

Advance registration is required and attendance will be limited because of space. For additional information write to: Registrar, Cold Spring Harbor Laboratory, P.O. Box 11724, Cold Spring Harbor, New York 11724, or call (516) 692-6660. W167(C)

To place your  
advertisement in these pages

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# applied excellence

## Advanced techniques in routine use at The Radiochemical Centre

Described below are just two examples of the many up-to-date techniques, which have been pioneered or applied for routine use at The Radiochemical Centre. These developments are part of our constant endeavour to maintain our position at the forefront of the specialised field of tracer methodology, so that we can continue our supply of radiochemicals of the highest quality and technical specifications.

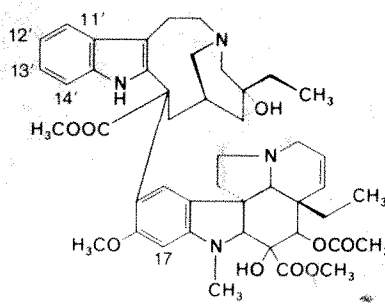
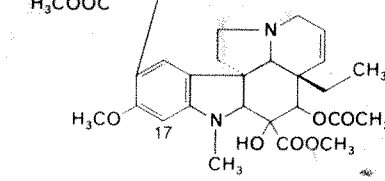
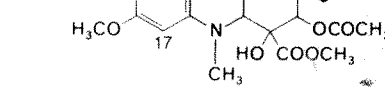
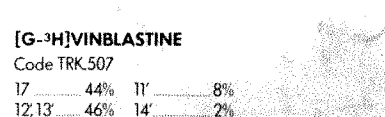
### Distribution of labelling in tritium compounds

Modern techniques for the production of tritiated compounds are more sophisticated than those used in the early days of tritium labelling, and produce compounds labelled in specific positions rather than generally labelled. Nevertheless, it is necessary for many tracer applications of tritium compounds to know the *precise* position and configuration of the tritium labels. Traditional chemical methods of doing this are tedious and time consuming and subject to considerable error, and so the routine supply of such information has until recently not been possible.

The Radiochemical Centre, in collaboration with the University of Surrey, has developed over the past eight years the technique of tritium nuclear magnetic resonance (tnmr) spectroscopy for this purpose. This method is much quicker and more accurate than the traditional chemical or biochemical methods for determining distribution of tritium labelling.

It is now used routinely to establish the distribution of tritium labelling produced by the usual methods of tritiation employed at The Radiochemical Centre. We supply accurate details as to the position and configuration of the tritium labels for an increasing number of our labelled compounds.

A number of examples are given below:

		<b>[18, 2β(n)-3H]TESTOSTERONE</b> Code TRK.162 1αc.....5.9%    2αc.....8.2% 1β.....46.0%    2β.....39.9%	
		<b>[6-3H]BENZO[α]PYRENE</b> Code TRK.501 6.....>95%    1.....<5%	
		<b>D-[2-3H]GLUCOSE</b> Code TRK.361 2(α).....40%    2(β).....60%	
		<b>L-[3,4(n)-3H]VALINE</b> Code TRA./K.533 2.....8%    4.....64% 3.....28%	
<b>[G-3H]VINBLASTINE</b> Code TRK.507 17.....44%    11'.....8% 12', 13'.....46%    14'.....2%			

The latest of our publications is as follows:  
 AL-RAWI, J.M.A., BLOXIDE, J.P., ELVIDGE, J.A., JONES, J.R.,  
 CHAMBERS, V.E.M., CHAMBERS, V.M.A. and EVANS, E.A.,  
 Steroids vol. 28 (3), p.p. 359-375, 1976.

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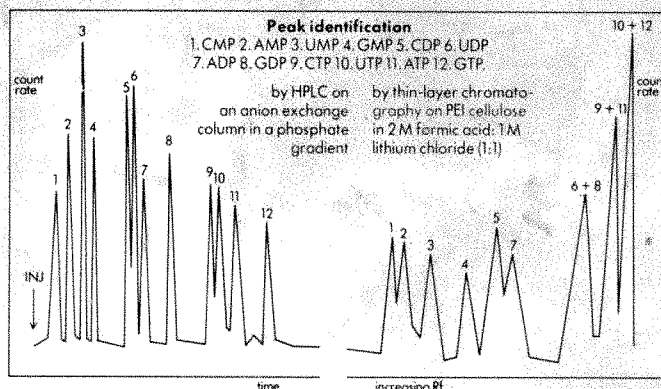
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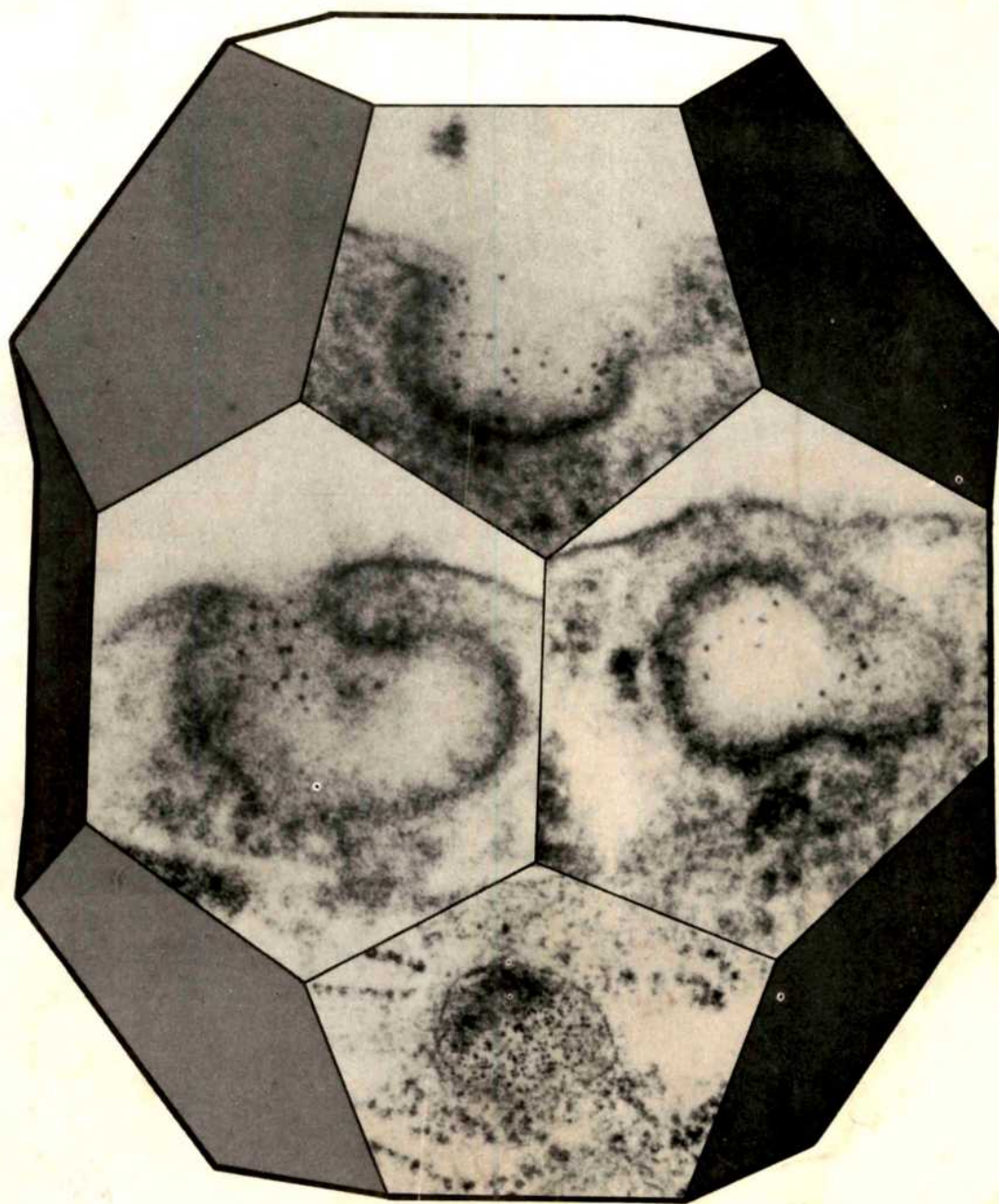


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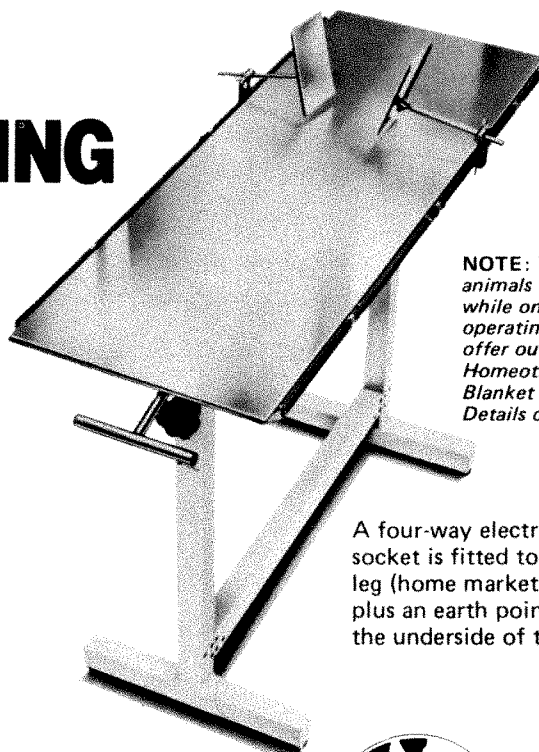
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A sequence of electron micrographs  
demonstrating the internalisation of  
low density lipoprotein into a  
coated pit. The general mechanisms  
involved are reviewed on pages 679–  
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● Articles may be up to 3,000 words long with at most six displayed items (figures and tables); they are reports of major research developments.

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**nature**

21 June 1979

## Cuts all round for UK science

In last week's *Nature* (page 565) we were editorialising about the growing weakness in support for R & D in the UK in comparison with other industrialised nations; this is not a weakness of resolve, it merely reflects a weak economy. The first budget of the new Conservative government underlines this weakness—expenditure in the higher education and science area is to be trimmed by between 1 and 1½ per cent as part of a very widespread operation to cut public expenditure.

The past two or three years have seen universities and research establishments pulling gradually away from the times of doubts and instability that characterised the mid-seventies. The University Grants Committee, charged with the responsibility for sustaining universities so that they have (among other things) 'well-found' laboratories in which research councils can support specific projects, has been able to make a little headway in replacing obsolete equipment; the UGC's equipment grant—of the order of £50 million—has even been growing in real terms by 10% annually. The research councils were pleasantly surprised late last year to pick up a promise from Mrs Shirley Williams, then Education Secretary, of £47 million additional cash over the next four years representing annual growth of around 3%. But now the prospects are not quite so bright.

The UGC has been successful in preventing the equipment grant from being cut (although universities will not be immune from the raising of VAT to 15%). But £9 million is being cut from the universities' recurrent grant and capital expenditure is also being trimmed. Nor is this all; there are several university wage settlements in the pipeline, and no guarantee that UGC will automatically receive the additional funds in full to pay for these settlements.

The research councils have had varied fortune. The Social Science Research Council has had nearly ten per cent of its funds taken away, the Agricultural Research Council next to nothing. The Science Research Council, by far the largest, loses £2.6 million of its budgeted £177 million for the present financial year, posing some threat to the 4.2 metre telescope now being planned for the new observatory in the Canaries.

The Medical Research Council might not seem to have come off too badly with a cut of just under 1% in its budget. It employs more people than any of the other research councils, however, and like the UGC will have to find money for increased salaries out of a reduced sum.

Scientists at the MRC units have been aware of this problem since early April when the previous government allowed a 5% increase in salaries in the unit's budgets for the 1979-80 financial year. Salary increases have been averaging at 15%, however, and many research units have been economising on new equipment whilst waiting to see what the new government had in store. With no increase in budgets for the units, the outlook was bleak; with a small cut it is bleaker still. Savings on equipment cannot continue indefinitely so posts will almost certainly be frozen and the already difficult problem of providing a reasonable career structure for scientists will be exacerbated.

It is clear that the cuts just announced do not represent any sort of detailed policy statement from the government on how it will pursue the support of science (with the possible exception of the social sciences). There is much nervous looking forward to the autumn, now thought to be the earliest time that the Department of Education and Science will reveal whether there are to be any major shifts in policy. There are relatively few straws in the wind, beyond that the government is involved in a 'quango' hunt at the moment. Quasi-autonomous non-governmental organisations (the UGC and research councils are amongst them) are said not to be terribly popular and there are moves afoot to trim them in various ways.

Those with memories of the way in which the Labour government of 1964 revamped the administration of science and technology will contrast the planning done by Labour in opposition with an apparent lack of preparation of science and technology policy by the Conservatives prior to this election, leading to a long period of waiting before policy surfaces. This is a disappointment; arguably science and technology policy is more important in 1979 than it was in 1964—even if there are few votes in it. □



# US Defense Department destroys evidence of Vietnam devastation

Alastair Hay reports on a row in the US over the loss of photographs used in the much disputed NAS report on the effects of herbicides during the Vietnam war

PHOTOGRAPHIC evidence, used by the US National Academy of Sciences (NAS) for its 1974 report on the effects of herbicides in South Vietnam has been destroyed by the Department of Defense (DoD). According to Dr Thomas Dashiell, staff specialist for chemical technology at the DoD, the photographs were destroyed "in late 1977 through the spring of 1978".

Estimates of the acreage of forest sprayed with herbicides and the number of trees killed as a result of the spraying were based on these aerial photographs. Hundreds of rolls of film for the period 1958-1973 were examined by the Academy in order to assess the extent of the damage. Dr Philip Handler, president of the NAS, told *Nature* that most of the photographs were "taken by or in co-operation with the Department of Defense and were studied at the College of Forest Resources, University of Washington".

Dr James Bethel, Dean of the College of Forest Resources, was in charge of that study. His initial estimate of "merchantable timber" killed by herbicides was considered too low by a review panel and the figure was revised upwards before the NAS report was published. The report estimated that some  $5 \times 10^5$  to  $2 \times 10^6$  cubic metres of merchantable timber had been killed out of a total of  $8.5 \times 10^6$  cubic metres sprayed. Spraying had in fact affected 10.3% of inland forests, 36.1% of mangrove forests, 3.2% of cultivated land and 5.5% of other vegetation.

Publication of these figures by the Academy created a storm of protest in scientific circles. Accusations that the estimates were too low were made by several scientists. Two members of the NAS committee reviewing the herbicide issue, Professor Paul Richards of the University College of North Wales and Professor Pham-Hoang Hô, University of Saigon, both dissented from the report on these grounds. Hô claimed that the estimates were out by an order of magnitude, a view which was supported by Professor Mathew Meselson of Harvard University and a member of the report's review panel. Meselson also claimed that the figures were 10 times too low.

The charges that the estimates were inaccurate were rejected by the NAS committee chairman, Professor Anton

Lang of Michigan State University. His reply to Hô's dissenting letter considered it "quite impossible" that the figures in the report could be out by the order of magnitude suggested.

Few NAS reports have created as much discord as this one. For this reason alone, many scientists argue that the evidence used by the NAS for the compilation of its report ought to have been kept. Dr Dashiell disagrees. He told *Nature* that the Department of Defense feels that "the NAS committee report represents a complete and well documented analysis of any ecological or environmental effects to the forests in South Vietnam". This, he says, was the principle reason for the study, and the photography provided for the committee study "represented only one means to reach their conclusions".

Dashiell says that the photography was kept for a longer period than originally envisaged. Following publication of the report, the photographs were placed in a repository at the University of Washington for use by scientists interested in doing further work on them.

This arrangement was approved by Dr Handler for the NAS, with the provision that a review be conducted after 12 months to determine whether the material be retained or destroyed. In the event, Dashiell points out, the material was left in the repository for two years, after which it was returned to the DoD where it remained for a further year before being destroyed. According to Dashiell, the scientific community "showed no interest during the lengthy time allowed for any review of their effort".

There is one scientist who was interested in these photographs and who is incensed about their destruction. Meselson, an outspoken critic of the NAS report, is in the process of compiling his own report about herbicide damage in Vietnam. In 1970, Meselson led a team of scientists to Vietnam on behalf of the American Association for the Advancement of Science (AAAS). Government officials in the White House, State and Defense Departments had advance warning of the team's preliminary report to the 1970 December meeting of the AAAS. A short time after this, an order was issued by the White House curtailing the use of



"It means 'Destroy Offensive Documents'!"

herbicides in Vietnam.

A summary of the AAAS findings were published in the Congressional Record on 3 March, 1972, but a final, fuller report of the team's investigations has never been written. Meselson has been chided in private on this point, but he told *Nature* that he hoped to be able to write the report this summer. One of the reasons for his delay was that he wanted to see the controversy surrounding the publication of the NAS report die down. This, he hoped, would give him an opportunity to produce his own report challenging the NAS estimates of timber damage on purely scientific grounds. Meselson makes no secret of the fact that the DoD's action in destroying the photographs has not made his job any easier.

Another reason advanced by the DoD justifying destruction of the photographs also seems rather lame. Dashiell insists that there were "physical storage problems" with the 35,000 items at the University of Washington repository so the decision was made to return the photography to the custody of the DoD. The story as related by Bethel at Washington University is slightly different. Bethel makes no reference to storage problems but says that, as no inquiries had been received about the photographs, he was "instructed by the Academy to return the photographs to the Department of Defense". According to Bethel, it was his understanding that the material was to be destroyed.

It seems, however, that although some probably irreplaceable scientific evidence has been destroyed, all is not lost; according to Dashiell, when the photographs were destroyed, the silver was recovered in accordance with "legislative mandates"! □

# California set to cash in on British discovery

LAST month the Scripps Clinic and Research Foundation in La Jolla, California, announced that it is going into partnership with the pharmaceutical company Miles Laboratories to provide laboratory testing services and prepare immunochemical materials for research scientists and biochemical manufacturers.

The new company will have a staff of about a dozen scientists and will also be able to call on the services of over 150 scientists at Scripps on a consultancy basis. It will offer a range of testing services and products in immunology, endocrinology and toxicology. And one of its most active fields promises to be the rapidly-growing area of monoclonal antibodies. These are antibodies produced by a technique developed a few years ago by Dr Cesar Milstein and Dr George Kohler at the Medical Research Council's Molecular Biology Laboratory in Cambridge, UK. It allows antibodies tailored to identify specific antigens to be produced in relatively large quantities, avoiding many of the uncertainties involved in conventional techniques of antibody production from animal serum.

Already the availability of these techniques and the resulting antibodies has led to what one observer describes as a virtual explosion of interest by research scientists. This has been paralleled, almost simultaneously, by the growth of commercial interest in what promises to be a lucrative range of applications. But although the original research was funded by the British taxpayer, for one reason or another, the National Research Development Corporation did not take out early patents on the techniques and the UK may therefore have forfeited any royalty rights which, some observers believe, could eventually have totalled hundreds of thousands of pounds.

The technique which the Cambridge scientists developed involved fusing two cells, one—taken, for example, from the spleen of a mouse—that produces a range of antibodies (but would be relatively short-lived) and the other a rapidly reproducing tumour cell. The resulting hybridoma cells are separated according to the particular antibodies that they produce, and each cell strain can be cloned to produce a pure source of a single antibody.

For scientists, the use of monoclonal antibodies as a labelling technique (since each antibody will only recognise a particular molecule) is proving to have a wide variety of uses. These range from studies of the structure of cell membranes, to the search for viruses that may be associated with the growth of tumours.

**Has Britain lost large potential royalties through a failure to recognise the commercial potential of antibodies?**

**David Dickson reports**

Commercial interest in potential applications has been equally swift. The most immediate applications lie in the field of screening, for example in the early detection of cancer, or of foetal abnormalities (since foetal cells can be identified at an early stage in the mother's blood by looking out for genetic material from the father). Other uses range from tissue typing to assisting in the search for new vaccines.

Recognising the great potential of monoclonal antibodies, many large medical instrument and pharmaceutical manufacturers are now eager to enter the field. Some have been working on the techniques in their own laboratories; others—as with Miles Laboratories—by tapping into the expertise of the academic community.

"The type of work which we will be doing in this field will be dictated by the needs of the complex of companies under the Bayer (parent company of Miles) umbrella, for which we will carry out research on a contract basis," says Dr Ernest Tucker, director of Scripps' Immunology Reference Laboratory, which will be a division of the new company.

"These companies will come to us with specific needs for antibodies. And since we are an independent company, we will be able to carry out research at the request of other companies too. It is much more efficient to do it this way, since the support of basic research is very expensive."

As well as established companies, the applications of monoclonal antibodies is also attracting the interest of the venture capital market, a booming business in the US following recent changes in capital gains tax and security laws. Like recombinant DNA research, it is a field ripe for swift marriages between sophisticated science and ambitious entrepreneurship but the pay-offs seem at present to be much closer to fruition, while at the research level at least, the field is relatively free of federal regulations.

A typical creation of the entrepreneurial spirit is Hybritech Inc., a company set up in San Diego earlier this year by the west coast venture capital firm Kleiner and Perkins (which also provided much of the initial capital for the genetic engineering firms Cetus and Genentech).

Hybritech has already announced

plans for producing and marketing antibodies for detecting hepatitis, a first foray into a clinical diagnostic market where current sales, according to Hybritech president Howard F. Greene, are around \$200 million a year, and expanding rapidly. Mr Greene predicts that the return on investment for successful research could be as much as five times higher than that in the electronics field. "Already about 90% of the major companies in the diagnostic business have called us, expressing an interest in our work. They are all at various stages of looking at programmes in this area, but the feedback seems to be that the large corporations are not moving with the speed and flexibility made possible by venture capital operations," Mr Greene told *Nature* last week.

The growth of demand for monoclonal antibodies has caused its own logistical problems within the scientific community. Many laboratories offer the hybridoma cells that they have developed to other research workers in the field on request, but this is consuming a large amount of time and energy.

Pressure is now growing for a centralised store of characterised hybridomas. Already a number of cell centres, such as the cell distribution centre at the Salk Institute in San Diego, have a growing number of such cells on their lists. Some scientists are now calling for a new centre, possibly financed by the NIH, to store and distribute hybridomas.

"What seems to be needed is a central depository which would maintain cells that have been carefully characterised and presented by research workers, thawing them out occasionally to check that the characterisation has been maintained," says Dr Hilary Koprowski of the Wistar Institute in Philadelphia. "The time seems ripe for a conference of people interested in the organisation of such a depository, deciding what types of cultures should be accepted, how they should be characterised, and so on."

One problem that a depository would have to face, however, is how it should deal with requests from private companies. With substantial profits looming on the horizon—"Somebody is going to make money in the diagnostic business from this technique," says Jerome Goldstein, head of the Clinical Assays Division of Baxter Travenol Laboratories—licensing of hybridomas promises to become a lucrative business.

At Stanford University in California, scientists who have provided hybridomas to Salk's Cell Distribution Center have required that any research



workers requesting a sample of the cells sign a waiver promising not to use the cells, or the antibodies produced from them, for commercial advantage. The university itself is currently negotiating with a number of pharmaceutical and medical instrument manufacturers on terms for supplying them with specific hybridomas and antibodies developed in the university laboratories. Dr Neil Reimers, head of the university's technology licensing office, sees that as providing the university with a steady

stream of income under an arrangement agreed with the National Institutes of Health, which provided much of the funding for the original research.

Whether or not Dr Milstein's original work in Cambridge could have been patented is still hotly disputed. Some claim that, as the application of standard cell fusion techniques is in wide use around the world, there is nothing inherently patentable about Dr Milstein's use of the hybridoma (although novel hybrid cell lines result-

ing from the techniques can, it is generally agreed, be patented).

Others, however, feel that as a sufficiently novel application of the techniques, a patent might indeed have been granted, requiring royalties to be paid to anyone using the techniques for commercial purposes.

But the net result is that, like penicillin and other similar stories, monoclonal antibodies may join the list of the ones that got away from the UK. □

## Italy's nuclear supremo goes for alternatives

The new chairman of Italy's nuclear energy committee plans to spend as much on alternative sources as nuclear power, writes **Robert Walgate**

For the first time in its history, the Italian National Committee for Nuclear Energy, CNEN, has received money to spend on the research development and promotion of alternative (non-nuclear) sources of energy. On 28 May, the outgoing coalition voted 5 billion lire (about £3 million) to CNEN to spend mainly on solar and wind power and on energy conservation.

The initiative is the work of CNEN's new Chairman, appointed on 1 February, Dr Umberto Colombo, previously research director of the firm Montedison. Colombo joined CNEN on condition that alternative sources would be included, and the 5 billion lire, he told *Nature* last week, is only the beginning.

"In Italy there has been no focal point for an energy policy" said Dr Colombo. "We want to become the focal point for all sources of energy which are alternatives to hydrocarbons."

At present, three ministers have partial responsibility for energy policy in Italy: the Minister for Industry supervises CNEN and the Ente Nazionale per l'Energia Elettrica (ENEL), which has responsibility for electricity supply; the Minister of Culture and Scientific Research directs the central body organising basic research in Italy, the Consiglio Nazionale delle Ricerche (CNR); and the Minister of State Participations has responsibility for the Ente Nazionale Idrocarburi (ENI) and the Istituto per la Ricostruzione Industriale (IRI); all of these being bodies with a more or less great interest in energy policy. The Minister for Industry has the largest responsibility—establishing the national energy



Colombo: "We might even be able to do without fast breeders"

plan, versions of which were published in 1975 and 1977.

"I want to make CNEN the entity responsible for all alternative sources, so that ENI remains responsible for fossil fuels, ENEL for electricity, and CNEN for R & D and promotion of nuclear and non-nuclear alternative sources, including the most important alternative—energy conservation."

The CNR is also undertaking an energy research project, but Colombo does not foresee conflict, rather co-ordination: "CNR is more involved universities; our contact is more with fundamental research and industry". ENEL and ENI have large geothermal projects "so we won't be able to do so much on that—probably we'll concentrate on hot rocks".

Dr Colombo hopes to make a substantial investment in non-nuclear alternative sources. The CNEN budget for 1979 is 200 billion lire (about £115 million), of which there is only 5 billion lire for alternatives; "but since we have only just started we could

hardly be expected to spend much this year". Next year, Colombo hopes to spend 30 billion lire (£17 million) on alternatives, and ultimately to reach an expenditure "very close to what we shall be spending on nuclear power".

Would it be possible to spend that much on new sources? "Well if you take some pioneer projects, and you consider that we are not interested so much in basic research as in demonstration units—in conservation, solar, wind, and so forth—it's possible". Clarifying the point, Dr Colombo said he has in mind an ultimate allocation of resources similar to that of the Department of Energy in the US, where nuclear—including fusion—still has more than other sources, but alternative sources have a substantial portion of the budget.

Nevertheless Colombo is convinced that nuclear power must make an increasing contribution to Italy's energy supply; at present one major and three minor reactors supply 0.7% of Italy's total energy needs and 2.7% of its electricity. By the year 2000, Colombo would like to see 20% of Italy's energy needs supplied by nuclear reactors requiring a building rate of two reactors per year; solar power, the main alternative contributor, might supply 7% by that date, he estimates.

"We do not have North Sea oil, we do not have the gigantic coal fields you have in the UK; at present we are very dependent on imported hydrocarbons. 85% of our energy is supplied by hydrocarbons, and 76% is imported energy. This dependence is not acceptable; we are very vulnerable. Our oil bill may go up this year, because of the price increases, by £1 billion; that's equivalent to exporting another million automobiles, if we want to keep our balance of payments in order . . . so a nuclear programme, as well as a vigorous programme on alternatives, is necessary for Italy."

Colombo makes a fairly classical connection between energy consump-



tion and economic growth. After conservation measures have been applied, he estimates Italy will be consuming 40% more energy in the year 2000 than now. "Don't measure that against UK standards. Our per capita consumption is 2.4 tonnes of oil equivalent; you have 3.7; so we must make great progress to reach other European countries. We can't possibly stay much behind them if we want to develop our own industry." The 40% increase corresponds to an annual rate of about 1.4%, and is based on an assumption of economic growth of 2.5% per year with an 'energy elasticity' (ratio between energy and economic growth rates) of some 50%.

Colombo is in the process of constructing his first 5-year plan for CNEN. "In the past everyone has been very superficial: both parliament and government, and CNEN, ENEL and the industry." Now Colombo believes that "we at CNEN must express our opinions forcefully, justify them with numbers and facts, and submit our ideas to a discussion in parliament." Then it is a matter of responding to the political will of parliament and government. Colombo hopes to submit his plan by the end of the summer.

Previously CNEN's energy policy was "very much in the air, something rather abstract". CNEN was moving in many different directions, in each of them "below the necessary threshold" of potential, manpower, and capital. Furthermore CNEN has been hampered by the government's inability to define a politically and publically acceptable nuclear plan. But in any case the previous plan "was too ambitious, and not credible at all".

"There were gigantic plans to build up a huge number of power stations, but the plans were never implemented. . . . They required almost a menu of different nuclear technologies, which—particularly after the Harrisburg incident—is no longer advisable. We need to concentrate on one type of proven reactor, and to put a great emphasis on the safety and security aspects of that one. I therefore will use my influence and my power—as far as possible—to determine this concentration of effort on one type of reactor. I do not think we have the technical or organisational capability to go ahead in many different directions in nuclear power in Italy."

The reactor will probably be the Westinghouse pressurised water reactor, which has also been chosen in France and Germany; this not because Colombo has any particular attachment to that but because the concentration of international effort and experience on it should make it one of the safest systems.

Nevertheless the Italian industry will continue to work on other reactor

systems, like the Tirrhena heavy water reactor presently under construction "to enable the industry to work on the commercialisation of CANDU-type reactors throughout the world". CNEN will also do direct and sponsored research and development of fast breeders and fusion reactors.

On nuclear safety, CNEN is at present both promoter and regulator of nuclear power—an unsatisfactory situation which Colombo expects to be changed sometime next year. At present "they both depend on me" says Colombo. But for the moment he is reluctant to give up the dual role. "We must first make the safety division of CNEN stronger technologically; I want to fortify it a little bit before giving it to somebody else. Secondly, the safety problem in nuclear plants is not just a health problem, it's a matter of checking the design, construction, and operation of very complex technological plants, which requires some technical expertise. If safety is left in the hands of medical people alone, and epidemiologists, they will cover very well the biological part but won't be competent in the other. So we need both, and we need to move towards the creation of an independent organisation. I hope it will be constituted under the premier, who controls both industry and health, allowing the organisation to include both types of competence".

"I am grateful to those who have been against nuclear power, although I do not share their ideas" said Colombo. "They have brought the problem to the surface, and obliged us to be more frank and serious in our considerations." In the past, he said, the nuclear authorities in Italy have been too arrogant in their dealings with the public; it is time to consider clearly and openly all the risks, both with and without nuclear power.

Furthermore, "if we are able to develop alternative sources immediately so that they can penetrate by 7% by the year 2000, and take off thereafter, we might even be able to do without fast breeders. But if the rate of penetration is slow, and if nuclear fission is the bridging energy source up to 2020–2030, we will have serious problems with uranium."

Colombo therefore wants to keep fast breeders open as an option, and Italy is already building pilot reprocessing plants "so that when we have to decide, we can decide from a strong position".

Ultimately, however, it is all up to the Italian government. "I'm powerful as far as propositions are concerned" says Colombo "but I'm not the one who decides". Colombo will be submitting his first 5-year plan to the government "in a month or two". □

## Energy efforts should shift from research to conservation

THE US Administration is placing too much emphasis on research into new energy technologies, and not enough into conservation techniques even though the latter have already produced considerable energy savings, according to a paper published by the Union of Concerned Scientists.

Dr Vince Taylor, the author of the paper, points out that improvements in efficiency made by industry since 1973 have contributed twice as much as oil obtained through the Alaska pipeline to the energy needs of the US last year. "90% or more of the solution to our energy problems will come from improvement in energy productivity—10% or less from supply extension. Yet the administrative and budgetary resources are being allocated in just the opposite proportions," Dr Taylor writes.

Dr Taylor lays out what he calls an 'easy path' strategy which, he claims, would redress imbalances in the energy budget by concentrating on factors such as improving the efficiency of motor vehicles and of energy use in domestic and commercial buildings, and selective switching between fuels. This strategy, he says, does not depend for its success on expensive and uncertain long-range R & D projects. □

## Carter proposes new fund to clear up chemical wastes

PRESIDENT Carter has submitted to Congress legislation setting up a \$1.6 billion fund for cleaning up hazardous chemical waste dumps and the effects of chemical and oil spills, in what one aide has referred to as "the most important environmental legislation" to be proposed by the Administration in the current year.

The President's move has been prompted both by general concerns about the effects of industrial pollution, and more specifically by a number of recent highly-publicised cases in which the previous dumping of toxic wastes has led to serious environmental and health hazards.

Against the advice of a number of federal agencies (although not the Environmental Protection Agency) the President has also decided that the industries responsible for the wastes should carry a large part of the bill for clearing them up. Consequently 80% of the fund would come through a series of fees imposed on oil refiners and chemical manufacturers. □



# Whitehall urged to relax grip on research funds

THE UK Agricultural Research Council is now poised to gain control of £2½ million of its funds previously tied to work commissioned by the Ministry of Agriculture, Fisheries and Food (MAFF). And this desire for greater freedom from the commitments of the Rothschild reorganisation of research has been reinforced by a plea from the Medical Research Council that Department of Health and Social Security control of its funds be reduced.

Both developments were revealed following last week's publication of the minutes of meetings of the House of Commons public accounts committee. In the case of the ARC it was stated that a review had been carried out by the MAFF and the Department of Education and Science under the previous government and this had investigated charges made by the ARC for use of its facilities. In particular, it was felt that more money should be paid by the MAFF for superannuation of staff carrying out its work and also for some capital costs. The full charges amounted to about £2½ million and it is now known that the Ministry has decided to pay this figure.

Although the MAFF will still control 54% of the ARC's budget, which last year totalled £45.6 million, it will now find £2½ million of its money earmarked for staff and other costs. This will free an equal sum for direct use by the council, which is likely to spend it on priority areas such as the genetic manipulation of plants with the aim of introducing new characteristics in crops.

However, the agreement to charge full economic costing for ARC facilities still has to be confirmed by the new Conservative administration, and this decision is expected shortly. Even when agreed, the effect will not be immediate, as many MAFF projects are being funded over long periods and there is no question of them being abandoned. But in the long term this financial reallocation will certainly give the ARC far greater flexibility in its undertakings.

In his plea to the committee, Dr James Gowans, the secretary of the MRC, said the transfer of its funds to DHSS control represented a vulnerable area in the council's £60 million budget: "It is vulnerable because the rules say the government departments are expected to spend the transferred funds with particular councils, but they do not have to."

He described the present arrangement as not being ideal—a dissatisfaction that stems, in part, from the 1976 cutbacks which resulted in the DHSS suddenly reducing its funding of

the council by 10%. "I should like to enter a plea that the MRC feels a little uneasy about the large chunks of its funds which could with political change be either decreased or might conceivably disappear. In fact, I should like to see in the long term the fraction of my council's total money, which is free money, increase—to give us greater stability and flexibility", he told the committee.

Dr Gowans said there were particular difficulties in trying to direct biomedical research through departmental contracts. One problem stemmed from the MRC's need to supervise all research, from basic to applied.

"In cancer, for example, we would



James Gowans, MRC: "vulnerable"

consider it essential to start with basic molecular biology and go right through to trials of new chemicals and radiation therapies", he added. It was of no use to pick a small fraction of a programme and concentrate only on that aspect.

"The other point is that if one looks back at the history of medical discovery, one finds that the key discoveries have all been made by accident", he stated. This also made it difficult to commission effective pieces of research and Dr Gowans indicated that he would prefer a system of saturating the very best talent with funds, although this would be hard to justify in times of financial stringency when many workers were competing for limited funds.

However, in his report to the committee, Sir James Hamilton, permanent secretary at the Department of Education and Science (which controls the major portion of the MRC's budget) said it would be wrong to suggest the idea of the customer-contractor relationship had failed—although he revealed that the DHSS-MRC relationship will be reviewed this autumn. This is to ensure basic objectives are being met in terms of scientific and financial accountability and are not being unnecessarily burdensome in bureaucratic terms.

Robin McKie

## WHO lays down safety guidelines

THE World Health Organisation is making progress with its attempt to lay down guidelines on occupational exposure to toxic substances. A meeting in Geneva this month agreed on a methodology for arriving at permissible levels of exposure to four heavy metals—cadmium, lead, manganese and mercury.

The first step is to examine all the available medical and scientific information, followed by the decision on what degree of risk can be tolerated. Degree of risk has to be decided at national level, partly because circumstances differ in some countries. For example, in the tropics, industrial workers may also be exposed to endemic diseases affecting the kidneys, liver or lungs. The acceptable level of metals toxicity affecting the same organs would therefore have to be much lower there than in northern industrialised countries.

The meeting made a breakthrough with agreement between the US and USSR on what should be the actual basis for exposure. The Soviets are considering switching to the American system of time-weighted exposure and abandoning their previous "maximum exposure" basis. There has also been

agreement on the principle of adverse health effects" for the four metals under consideration. Two criteria have been laid down: (a) is the effect reversible or not? (b) are compensatory mechanisms in the body impaired? From this it is possible to define the effects of each substance on an agreed "target organ".

Finally, the Geneva meeting agreed on permissible levels for each metal, both "biological levels" in the person affected and atmospheric levels in the place of work or in the immediate vicinity of a plant such as a lead smelter.

The need for guidelines such as these was because of the wide discrepancies in permissible levels between various industrialised countries and the feeling that guidance would help Third World countries moving into industrialisation. Previous attempts to recommend levels of toxicity through the International Labour Organisation have tended to be blocked by employers pressure groups. The WHO approach was specifically medical and scientific, but it is understood there were, nonetheless, messages of strong disapproval from a number of industrial firms.

Peter Collins



## news in brief

**Sussex students occupy physics building:** The protest at Sussex University (14 June, page 568) continued to escalate as students occupied the physics building, which houses the university's main telephone exchange. The telephone service to the university was cut off. The occupation, which began on 15 June and is expected to last for at least another week, is the result of a week's failed negotiations. Students elected the "excluded" student body president Richard Flint and engineering student Shaun Fensom, to present their proposals for joint talks with staff at the university Senate meeting. (Their proposal has been widened to include the case of 305 students threatened with academic sanctions because of a current rent strike against university housing. The students voted that sanctions or threats of sanctions should be withdrawn as a precondition for talks and voted to go into occupation if the amnesty proposal was rejected.

Upon seeing the two excluded students in the meeting hall, Vice-chancellor Denys Wilkinson called off the Senate meeting but was persuaded by moderate staff to reconvene it. A morning session was followed by a massive rejection in the afternoon of the student proposal. There are no immediate plans to call off the occupation and administrative hopes that the summer vacation would dampen opposition were dashed as the students voted to disrupt conferences during the summer at their last general meeting on 19 June.

**Philippines call halt to nuclear plant:** The Philippine government has halted construction work on a £600m Westinghouse reactor for alleged violations of the safety warranty. The plant was begun in 1976 and was to be finished in 1983. The government acted when Westinghouse refused to send a safety team to the Philippines to discuss the Three Mile Island accident, and after the US Union of Concerned Scientists had sent a detailed plan of deficiencies in engineering and design. Westinghouse had commissioned an independent inquiry which found that there was no evidence that the reactor would be unsafe. The ban on building will not be lifted until the government is convinced that the plant posed "no danger to the people."

**Vietnam veterans studied for herbicide exposure:** Vietnam veterans are to be the subject of a study of the long term effects of exposure to the herbicide, 2,4,5-T. The investigation, ordered by the Carter administration, will examine 1,200 veterans exposed to the herbicide during spraying missions over Vietnam in the years 1962-1970. Scheduled to last six years, the investigation will be carried out by the Air Force and the Department of Health, Education and Welfare; it will supplement an inquiry already begun by the Veterans Administration. Concern about the health hazards of 2,4,5-T and its tetrachlorodibenzo-p-dioxin contaminant—a known teratogen and carcinogen—has increased in recent years. Vietnam veterans were exposed to the herbicide in a formulation called Agent Orange of which 2,4,5-T is one of two components. Agent Orange was known to have much higher concentrations of the dioxin than 2,4,5-T preparations in current use.

Clinical data from the 1,200 subjects will be compared with that from a control group of 1,800. Doubts still remain about the scientific methods to be used in the study. Evaluation of the results will also prove difficult with the wide ranging clinical symptoms attributed to exposure to 2,4,5-T. Veterans are claiming that psychological disturbances, malaise and death from cancer can all be traced to exposure to 2,4,5-T.—*from Alastair Hay.*



**New HSE microbiological adviser to be tough on safety:** The UK Health and Safety Executive has appointed Dr Bob Harris (left) as its adviser on microbiological hazards at work. Harris, director of the former Microbiological Research Establishment at Porton Down, is a firm believer that society must control the safety of research work. He also supports trade union and general public representation on committees such as the Genetic Manipulation Advisory Committee. Harris took up his post of senior consultant adviser on 21 May. He will oversee the development of contaminant assays and training of HSE staff in their use, and he will represent the HSE on committees and public bodies. Harris is an experienced cancer researcher, most recently as head of the Imperial Cancer Research Fund's Division of Experimental Biology and Virology. His appointment is expected to silence criticism from "a minority of researchers" who have opposed HSE interventions in laboratory safety.

**British delegation tries to sell Magnox reactors in Peking:**

A British Energy Exhibition organised by the UK Department of Energy and the British Overseas Trade Board visited Peking from 6-16 June to sell energy technology to the Chinese. British Nuclear Fuels Ltd and the Nuclear Power Company are trying to interest the Chinese in the UK's Magnox reactor, an old first generation machine that is considered to be the safest nuclear reactor. Mr Robert O'Neil, head of engineering safety and technology at the UK Atomic Energy Authority, gave a long talk on reactor safety to a Peking conference on 12 June. O'Neil told the Peking audience that the probability of receiving a lethal dose of radiation at a distance of 15 km from a reactor was approximately the same as being hit by a meteorite or "killed in a supernova explosion of a star". There is no record yet of specific questions asked by the Chinese about reactor safety.

**Environmental agency urges destruction of nerve gas bombs:**

The US Environmental Protection Agency has recommended to the Defense Department that it should destroy the Army's stock of 900 Weteye nerve gas bombs, rather than move them from a Denver arsenal to a base in Utah. This follows evidence that a number of bombs are leaking inside their sealed containers.

Although the Army had initially announced its own plans to destroy the weapons (the most modern chemical weapons in the US armoury produced shortly before a ban was imposed by President Nixon in 1969). Defense Secretary Harold Brown announced last year that the weapons would be kept as a deterrent to any use of lethal gas in war by the Soviet Union. Plans to move the bombs, which contain more than 300,000 pounds of gas, were first proposed for last year, but postponed when two were found to be leaking. Following a new announcement last month by the Defense Department that it would shortly begin transporting the bombs after an investigation had concluded that the rest of the bombs were safe and could be moved, Utah governor Scott Matheson announced his intention to seek a court order preventing their transport.

In a letter to the officials of the Rocky Mountain Arsenal, where the bombs are currently housed, the EPA says that, in view of newly-discovered leaks, the bombs should be destroyed.



# CERN—the next 25 years

The European centre for subnuclear physics—CERN—celebrates its 25th anniversary next week. But it is a time for Europe to look forward, not back. **Robert Walgate** reports

AMERICAN high energy physics laboratories—there are three major ones—have generated one or two Nobel prizes, and much spectacular physics. On the other hand CERN, the European centre for high energy physics, has discovered 'neutral currents' (a new kind of weak interaction), but nothing else with the feel of a Nobel prize. (The one for neutral currents hasn't been awarded yet.) CERN has been much praised for the quality of its work; but sometimes this has felt like being patted on the back for trying hard. However, all this seems about to change.

Burton Richer, an American who won a Nobel prize for discovering the  $J/\psi$  (indirectly a discovery of the charmed quark) agreed last week that in the last 25 years the greatest discoveries have been American; but in the next 25, he said, "Europe will become the senior partner". And according to Leon Lederman, also a man of spectacular discoveries (he recently found the next quark, bottom, in a 9.5 GeV particle called  $\psi$ ), "the US is going to have a rather hard time competing".

However "it's nonsense" says Lederman, to say that CERN was too concerned with quality and missed the spectacular results. "Anyone can get spectacular results". The reason the US got most of the big discoveries was partly the statistics of small numbers—luck. "Plus we had a heritage of experience. We had accelerators running in 1951." CERN's first, the SC, started up in 1957. And now he envies the quality of CERN engineers. "The European engineering education is better based in mathematics and physics and ideally suited to accelerators."

Lederman should know, for it is, in some sense, his job to compete: he has been appointed director of The Fermi National Accelerator Laboratory (Fermilab) in Chicago, America's nearest equivalent to CERN. But he says "we're undermanned by a factor of 2 or 3; we don't have the people or the money". The previous director, Robert Wilson, resigned over a question of funding.

But CERN isn't going ahead simply because the US is falling behind. It has three adventurous plans on the books: one approved and building; one on the verge of approval; and one very ambitious scheme yet to be put to the political test.

The first (the antiproton collider) should generate physics from the collisions of protons and antiprotons within the present 500 GeV accelerator ring towards the end of 1981. There's probably a Nobel prize in it through the discovery of the 'intermediate vector boson' (a particle as important to modern physics as the structure of DNA was to 1950s biology). The second (LEAR) should use stored antiprotons for unique experiments in low energy nuclear physics. And the third (LEP) would generate 200 GeV collisions between electrons and positrons (presently the cleanest way of investigating the fine structure of matter) towards the end of next decade. There are probably a few Nobel prizes in that.

A firm and unanimous proposal for LEP, the result of the deliberations of hundreds of physicists throughout Europe in the technical panels of ECFA (the European Committee for Future Accelerators), should go to CERN Council on 9 November this year. LEP is CERN's future for the late 1980s and 1990s; it is up to governments to decide if CERN can have it. (The clash between Germany and CERN over the siting of LEP has died down; CERN will have it, while Germany's national Hamburg laboratory DESY will add protons to its electron rings: at least that's the physicists' plan.)

Hamburg, too, is ahead; experiments are underway there on what is presently the world's highest energy electron-positron machine, PETRA, with the American equivalent, PEP (Burton Richter's machine), coming on in November about a year later because of funding delays.

But America hasn't given up. According to Lederman "I think Fermilab is going to cause CERN a lot of grief". Fermilab is building—and has been building for some time—a ring of superconducting magnets (the 'energy doubler') to join the normal magnets in its 500 GeV proton accelerator tunnel, and these magnets will give Fermilab the capability of reaching beam energies of 1000 GeV to collide with a target, or making collisions between 1000 GeV and 1000 GeV counter-moving protons. Lederman hopes to phase in physics on the colliding beams by 1982; and on fixed target by 1983. This would give Fermilab the highest energies in the world. "The doubler is the only way



Leon Van Hove, CERN research director: leaving "after a very interesting period of defining future projects"

we can get ahead" says Lederman.

(Ultimately the USSR should overtake Fermilab with UNK, a 10,000 GeV—10 TeV—machine.)

Furthermore at Hamburg there have been problems in reaching design collision rates (which determine whether an experiment can be done in months or years) because of a complicated acceleration system which will not trouble PEP. Richter says "I've laid a number of bets that within two weeks of first injection we will be at 10% of design luminosity". PETRA has been sitting around 1%, and although this figure is rising fast there will be a lot of physics left for PEP to do.

A few years ago such competitiveness was justified in terms of repeating experiments: if Fermilab discovered something, then CERN ought to check it. There was an example of that recently, when an anomaly in neutrino interactions discovered in a rough and ready fashion at Fermilab was checked—and discounted—in a more sophisticated experiment at CERN.

## Sharing unique facilities

But the future seems to be one of unique, continental facilities and no overlap.

So a number of physicists—particularly American ones—foresee a period when there will have to be increased international cooperation, even going so far as a transatlantic pooling of funds on projects such as, for example, the energy doubler at Fermilab. But according to the chairman of ECFA, Marcel Vivargent, this is impossible at present; CERN funds are very rigidly controlled by the member states and must be spent only for Europe. "But if LEP is accepted"

said Vivargent last week "we will have to discuss cooperation".

Leon Lederman, Fermilab director, raises an interesting issue: there are 26 European groups currently working at Fermilab, he says, who have come through the usual selection procedure by merit. "We have no way of assessing where a proposal comes from . . . but the energy doubler is going to raise a question of discrimination." With their (disputed) better funding the European groups will have better equipment for their experiments, he says, and without discrimination this would leave the Americans in the cold. There seems to be a possibility here that if competition does not ease, it will increase. No sharing of funds, no sharing of experiments, may be a new threat from the US to Europe.

Leon Van Hove, research director-general of CERN, feels that the normal exchange of groups (evidenced by the 26 European groups at Fermilab) will suffice; and that although exchange is increasing, no new procedure or institution (to match groups and laboratories) is necessary. The International Committee for the Future of Accelerators (ICFA) under the International Council of Scientific Unions (ICSU) would be sufficient to discuss the matter, he feels.

Burton Richter, on the other hand, believes that the old ways will not suffice, because of the large and increasing cost of the apparatus that must be built up around an accelerator to do a particular experiment. A single detector at LEP would cost about \$20 million, he estimates; who is going to pay for the apparatus, the group or the laboratory?

And how should the operating costs of the accelerators be divided? According to Lederman, Fermilab's electricity bill is presently \$7 million, and may rise to \$10-12 million next year (though the superconducting energy doubler, used not to double beam energy but to save accelerator power, could cut the costs 70%). And LEP, according to some estimates, if it used conventional magnets, could consume 500 MW of electrical power. (But that estimate is sensitive to the assumed radius, and to the energy of the machine. The consumption rises as the fourth power of the beam energy.) With costs and consumptions such as these, intercontinental cost-sharing may be necessary.

Whatever agreements may prove to be necessary on this level, there is a definite feeling both in Europe and the US that Europe now has the initiative. In the short term the US has PEP, which will be competitive with PETRA; in the long term, the American future depends on the

technology of superconducting magnets, which Fermilab is still wrestling with. (Another US machine for the mid-80s, Isabelle, a high energy East Coast machine to collide protons with protons, also needs superconducting magnets.) Lederman says Fermilab is about to go into a 1,000 magnet production run (they have produced 200 in various batches so far) but CERN cynics say they have heard that before; the technology is proving very difficult.

(If LEP needs superconducting accelerating cavities to save power costs and reduce that 500 MW to something manageable, it will face a problem of even greater magnitude. But test cavities are already in construction at Karlsruhe in West Germany for trials at DESY.)

Is there a simple reason why Europe appears to have taken the lead? According to Lederman, funding has a lot to do with it. "In the early 50s we were better funded, but somewhere in the 70s the budgets crossed." In CERN, it is not felt that Europe has much more money to spend—rather that the US is stretching its resources across too many projects. "If we had the energy doubler, PEP, and Isabelle, I wouldn't say we badly off" said one CERN physicist. And according to Leon Van Hove "to run three big labs on a small budget is trying to square the circle".

### Delayed advantage

Also, the delay in building the 500 GeV super proton synchrotron (the SPS) at CERN may, in retrospect, have been an advantage. While the US committed a large fraction of its funds to the construction of Isabelle, which many now see to be an outdated machine, it became clear that a major way forward in physics was to collide particles with their antiparticles (electrons with positrons, which gave us charm, or protons with antiprotons). Hence when the SPS was built, European physicists were free to use their new experience in allocating the potential European budget to the antiproton collider and to LEP.

Furthermore there appears to be a new mood in CERN. It used to be accused of being very strong on engineering—accelerator design—but unadventurous on physics. There was a strong division between the accelerator people who wanted a beautiful machine, and the users who wanted physics quickly. Now that divide is being bridged, and the accelerators are really being designed to suit the physicists, while the physicists are becoming more aware of the real constraints of design. LEP is certainly benefiting from this, and so is the antiproton collider.

That particular project is very instructive. An Italian with Italian temperament and American determination, Carlo Rubbia, who had been doing neutrino experiments at Fermilab and making a few discoveries (dimuons, events now connected with charm, and the anomaly later discounted at CERN), decided that the Fermilab and CERN accelerators could be used to make the intermediate vector boson (this latest holy grail of physics) if antiprotons could be injected into them the wrong way round. Simple in principle, but difficult in practice; but Rubbia was enough of an accelerator engineer to make some sensible proposals. He made them, to CERN and Fermilab, around 1976, and from then on the labs were in competition.

Then a number of interesting events occurred. First, it became clear that the vacuum in the Fermilab machine, at  $10^{-7}$  Torr, while good enough for the accelerator, would not do for day-long storage of intense beams (beams were lost in 30 minutes); while the SPS, over-designed to some American tastes at  $10^{-8}$  Torr and in easy reach of  $10^{-9}$  Torr, could do the job it had not been designed for.

Second, the CERN team in charge of future projects rejected the scheme, which involve creating antiprotons, catching them, and 'cooling' them into a confined beam, as impractical—so the project was taken on directly by people who were in favour of it. "There were people at CERN who were determined not to be beaten again" said one member of Rubbia's group. "They'd lost the J/psi and the upsilon to the US and they were not going to lose the IVB". There was, in fact, a kind of breakthrough by radical elements, greatly assisted by the persuasive powers of the antiproton advocate, Carlo Rubbia.

Third, the CERN directorate swung wholeheartedly behind the scheme (in fact they had a choice between two radical proposals, one involving electrons; it's arguable which was best) and put a good deal of CERN money into it.

And finally, the CERN accelerator physicists and engineers moved rapidly in reaching the solution of a very difficult technical problem, and proving it in a beautiful experiment, the initial cooling experiment (ICE). The project drew on all available CERN expertise, but decisions were taken sequentially as step after step was proved. There are still two years and many problems to go, but the antiproton projects appears to be CERN at its very best—using not only its skills, but also a large dash of imagination. Yes, Professor Lederman, it's going to be a very difficult combination to beat. □

# Science in Bolivia: the great divide

Bolivia has had 190 Presidents in 150 years of independence. Seven out of 10 of its poverty-stricken people are illiterate, and yet the country has eight 'liberal' universities for the privileged few, generally those of European descent.

This frustrating formula is carried over into science: research is confused, geared to foreign backers and largely unrelated to the needs of the people.

**John Hutton**, from the Department of Clinical Biochemistry at Addenbrooke's Hospital, Cambridge, visited Bolivia on a Nature writing fellowship.

THE village of Patacamaya lies to one side of the road that links the commercial centre of Bolivia, La Paz, with the mining town of Oruro. To the outsider, it is just like any other of the windswept settlements scattered across the Altiplano, the high plateau where 60% of Bolivia's five million people live. The village square seems deserted, the adobe huts practically uninhabited. There is a pervasive air of forlornness, only dispelled when the first light appears on a Saturday. Before dawn, trucks that have arrived from all over the countryside crowd into the marketplace, stacked with produce and people. Salt blocks conveyed by llama trains from the salars to the south west are exchanged for fresh fruit and vegetables transported across the mountains from the jungle regions to the east. What money changes hands is spent on a few manufactured goods from La Paz or a farming implement forged in the open air foundry beneath the eyes of the crowd. In the late morning it is time to celebrate the purchase of a cow, perhaps, or consult with a herbalist, but by the afternoon the dust has settled and the stillness has returned.

Less than seven kilometres from the village, and in stark contrast, is a Ministry of Agriculture research station, a monument to the agricultural potential of the high plateau. Established on what was formerly an estate thought too poor for redistribution under the agrarian reform of 1953, it demonstrates the transformation that can be wrought by the application of machinery, fertilisers, and plant and animal breeding techniques. To the Indian farmer tracing a furrow behind a single-bladed wooden plough tethered to a pair of oxen or to the shepherd following his few llamas and sheep as

they seek sustenance amid the stones and stubble nearby, the research station seems no more of his world than is the Russian satellite tracking station also located at Patacamaya. If anything, it only serves to remind him that he stands outside the money economy, that nobody cares for his education, that some of his children will die before reaching adulthood and that none of his family can expect to live for more than fifty years. Although the revolution has given the native farmer back his land, it seems that the administration has no place for him. With respect to health and education, the rural dweller is ignored, left to eke out a subsistence living from an inhospitable environment with little but his own meagre resources.

Somewhere between the agronomist of the research station and the subsistence farmer there is another man: a farmer's son, perhaps, whose primary education at the local school sets him apart, and who sees no future for himself in the bleak countryside. Given the opportunity, he would make the journey to La Paz, in the hope of becoming a gardener or a servant, or to work in one of the few factories. In time he might hope to own a truck or to rent a stall in the sprawling marketplace, and his dreams would be to change his traditional habits, purchase a few goods such as a radio or television and to educate his children into a better place in society.

The present Bolivian educational system, which leaves 70% of the population illiterate, still finds a place for eight universities—despite the country's small population. Amongst them is the oldest university on the South American mainland, the Royal and Pontifical Higher University of San Francisco Xavier of Chuquisaca. Traditionally, these institutions served to maintain the Hispanic cultural heritage and they were dedicated to the



formation of a liberal professional elite, whose ranks excluded both women and the native population.

Today, amalgamated under the title of the Bolivian University, they are nominally non-sectarian, academically autonomous and open to all. Despite this, membership has generally remained the privilege of an urban class of European or mixed European extraction. Enrolments in economics, law, medicine and engineering still account for more than half the student population, and so the country has too many doctors and too many planners who, in the absence of appropriate jobs at home, are forced to seek employment overseas.

The pursuit of science, as a career leading to technical expertise or as a research discipline, is invested with little of the prestige attached to the liberal professions. University teaching is hampered by the national political instability which, combined with the volatility of the student population, can result in the suspension of classes at any time for periods of several months. The majority of teachers have little more than a basic degree or may even be unqualified, having obtained their position through personal influence. Even those with full-time positions often work at a second job, leaving their classes to one of their assistants, usually a final year student. In science, practical training is limited as there are often no laboratory facilities, or materials are restricted to what the student can provide from his own funds. Postgraduate education does not exist at present, but even if the plans for it were implemented, who would be the teachers? In short, the few students who wish to become scientists have little choice but to seek their training overseas.

Upon their return they may find limited opportunity for research except in the fields of cosmic ray physics or

*Drawings by Professor P. J. Schofield of the University of New South Wales, Australia*



high altitude physiology. Bolivia's geographical position, with a range of altitudes up to 7,000 metres above sea level and a large proportion of the population living at around 4,000 metres, provides an ideal laboratory for this type of research and has attracted investment from overseas in establishing research facilities in these areas.

Yet even here an overseas-trained scientist does not always find himself in a hospitable environment. His newly won diplomas may not be recognised, he may find difficulty in getting on to the university payroll and he will inevitably encounter pressure from his peers to conform to the sedentary existence of preparing reports or feasibility studies rather than hazard the difficulties of performing a concrete task. While his knowledge of his discipline may be superior to that of his colleagues, his adroitness and experience in the political realm may not match their skills. If this is the case, he may be lost. The few scientists who do succeed in establishing themselves in a productive research career do not always remain there. Summary dismissal for political reasons may terminate their work, or, by a more insidious process, they may find their efforts spread over too many projects, and their energies dissipated by travel overseas to consult upon the development of their country.

A better climate for science seemed in store at the end of 1977, when President Banzer established by supreme decree a Council of Science and Technology with the powers to

assess and direct scientific research within the country. This was the first time that any Bolivian government had recognised that current research could be of use, and that further investment in research might assist in the development of the country. The council has not, however, received a great deal of financial support, and it may be only a gesture in response to pressure from the Organisation of American States and the United Nations. Nevertheless, despite two changes in the military government and the usual administrative reshuffling that accompanied these, it manages to survive and is now making its plans known.

The scheme of development which is envisaged in the council's early reports is implicitly that of the construction of a Western-styled, capital-based, consumer society. Progress towards this is to be achieved by the further exploitation of mineral resources, the production of cash crops for the export market and the expansion of industry with the aid of foreign technology. The scientist is seen as the source of new technologies which will help relieve the stranglehold imposed by foreign corporations. The council sees the promotion and funding of local research, however, as the responsibility of industry, or of the developed world through the establishment of an international fund. But what investor is going to be so benevolent as to buy his way deliberately out of a market? Institutions such as the International Development Bank, after all, do not invest their money for love.

In keeping with the council's broadly defined goals, steps have been taken through the universities and their associated institutions to support on-going research which has the potential for immediate economic impact, and to encourage specific projects of an applied nature. Such changes are to be made within the framework of the present budgetary restraints, a move which seems to lead inevitably to the demise of cosmic ray physics and high altitude physiology. On the other hand, the futility of supporting too many specific projects is revealed by a glance at any of the laboratories throughout the country, many of which house elaborate equipment that has never been operated or even installed. The situation is invariable: the initial project grant failed to account for the services of a skilled technician, a part was missing or an essential chemical not ordered, and by the time funds were available again and the deficiencies made good, time had passed, enthusiasm waned and the research team disbanded.

The agricultural research station at Patacamaya is one project which has fulfilled its aims. But the agronomists who are bent on emulating an agricultural system designed to suit another physical and economic environment often find it difficult to see past the superstition and ignorance that surrounds them. Even if they attempted to make contact with their neighbours, the chances are that there would not be a common language in which to communicate their ideas. The agro-



Farm tools for sale in La Paz market



"A pervasive air of forlornness . . ."—pictures by John Hutton



nomist may speak only Spanish, the sole idiom in which education is available, while the native farmer may speak only Aymara, the native tongue of the high plateau. Disillusionment is mutual. The frustrated scientist may well question the validity of sophisticated techniques such as ova transplantation or investigation into animal genetics when there seems to be little scope for the implementation of this kind of technology. No wonder that his sharper colleagues have taken office jobs in La Paz or that almost half the class with whom he graduated have left Bolivia permanently.

The experience of other Latin American countries suggests that investment into applied research only is in fact a costly and inefficient path to the development of new technologies. It is important for these countries to



encourage basic research, develop teaching standards and invest in library facilities: in sum, to construct the base of an indigenous establishment which could eventually define and resolve scientific problems within the context of the society.

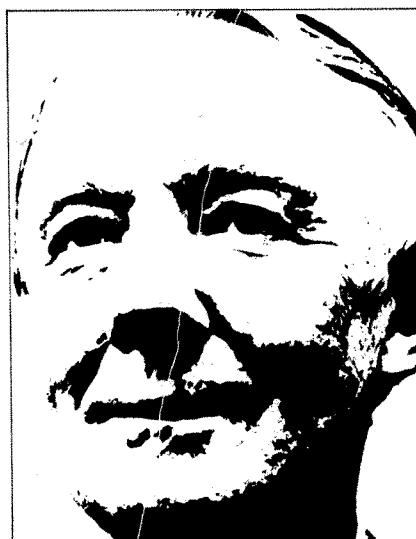
In a country which has seen more than 190 presidents in the 150 years since colonial rule ended, and where government is largely a question of survival, a change of heart is essential if a longer view of investment in scientific research is to be propagated. Meanwhile, Bolivia has few safeguards against the encroachment of modernity and almost no inherent means of curbing the aspirations of a ruthless dictator or foreign investor. Given the present state of the society, it seems that Bolivian science and technology will remain the artefacts of the more developed powers. One wonders, then, how much longer will the agronomist and the native farmer, each surrounded by the trappings of his own culture, continue to gaze uncomprehendingly at each other's flocks across the fence? □

## Double standards

TWENTY years ago in Britain there was considerable concern that pesticides might wipe out much of our wildlife. Thousands of corpses of seed eating birds were found in the cornfields of East Anglia, poisoned by aldrin or dieldrin, used as a seed dressing to protect the newly-planted grain from attack by the wheat bulb fly. Hawks which preyed on these seed eaters, and foxes and badgers which ate their corpses, were also affected, and the population of the Peregrine falcon was eliminated from many parts of the country. When the cause of this holocaust was recognised, British farmers and pesticide manufacturers agreed in 1962 to a voluntary ban on the more dangerous use of these pesticides, and since then the situation has greatly improved. The slaughter of seed eating birds has ceased, and the populations of predators have increased considerably. There are still isolated incidents when pesticides kill numbers of birds and other animals, usually arising from the misuse of the chemicals, but in general the situation is satisfactory.

This improvement in Britain occurred as a result of the cooperation of naturalists, scientists and industry. Bodies like the voluntary organisations of ornithologists, the Royal Society for the Protection of Birds and the British Trust for Ornithology, worked closely with the government supported Nature Conservancy and the official Ministry of Agriculture to spell out the recommendations of the Pesticides Safety Protection scheme with the members of the chemical industry. It is perhaps significant that their successful regulations were brought into force some time before the appearance of Rachel Carson's book, *Silent Spring*, which is often thought to have alerted people to the danger of pesticides. Scientists had been well aware of the dangers for several years, and, as I have indicated, had done something practical about them. I fear that the main effect of *Silent Spring* was to make the uninformed over-react, and to deny the safe use of beneficial chemicals to many people throughout the world.

However, the situation varies from country to country. It has often been alleged that while the chemical industry has been very responsible in developed countries like Britain and other parts of Western Europe, where they have restricted their sales of chemicals known to endanger wildlife and the environment, they have behaved quite differently overseas. Up



KENNETH MELLANBY

till now I have seen little evidence that they are dumping chemicals abroad that they cannot sell in western countries. However, recently on the shores of Lake Titicaca, 3,812 m above sea level in the Peruvian Andes, I saw a huge, ugly hoarding advertising aldrin on behalf of the Shell company. Quite apart from the visual pollution of a beautiful previously quite unspoiled landscape, I doubt the wisdom of this commercial enterprise. I also saw, in the offices of the government agricultural officer at Junin, only a few kilometres from the even higher Lake Junin (4,081 m), a pictorial calendar advocating the use of a whole battery of organochlorine pesticides the use of which is strictly controlled or even banned in most developed countries.

I would be the first to agree that medical and dietary problems in developing countries are such that pesticides are essential, and that to save human lives wildlife may sometimes have to be put at risk. With skilled operatives, there may be a place in Peru for aldrin. But all countries, developed and developing, need a code of practice something like that so well observed by industry in Britain. If substantial amounts of aldrin get into the hands of the unsophisticated peasant farmers of the high Andes, the chemical is bound to find its way into the lakes and rivers. There it could well be concentrated in the bodies of the fish and other aquatic life, and could then be passed along the food chain to the unique birdlife of the region. The result could be an ecological disaster. The economic gains to the chemical industry or to the farmers are surely not sufficient to justify such risks.

# news and views

## Why do messengers wear caps?

from M. J. Clemens

DURING the past decade we have learned a great deal about the structures of messenger RNA molecules in both bacterial and higher cells. Pains-taking work in several laboratories has brought molecular biologists from a position of doubting whether such entities really existed to one of considering which particular structural features enable these molecules to function as the efficient carriers of genetic information in living cells. One of the most exciting (and unexpected) developments in this story was the discovery of the 'cap' structures at the 5' ends of most eukaryotic (but not bacterial) mRNAs. These caps comprise a methylated guanosine residue linked by a 5'-5' triphosphate group to the first coded nucleotide of the RNA; the latter, and the subsequent nucleotide in the sequence, can also be methylated, giving rise to structures of the type  $m^7G(5')ppp(5')X^m pY^m$ .

There is now a considerable amount of information concerning the biosynthesis and the functions of cap structures in eukaryotic cell and viral mRNAs (reviewed by Shatkin *Cell* **9**, 645; 1976 and more recently by Filipowicz *FEBS Lett.* **96**, 1; 1978). Several possible roles for caps have been proposed, including stabilisation of mRNAs against degradation by 5'-exoribonucleases. Greatest attention, however, has been paid to the influence of the structures on the efficiencies of translation of mRNAs. Several complementary studies, which have included the use of uncapped and capped molecules as templates for cell-free protein synthesis, and investigation of the effects of compounds which resemble the cap structures, have revealed that the presence of a cap usually enhances the ability of a messenger to code for a polypeptide chain. In the cases where the mechanism of this enhancement has been investigated, it appears that capped mRNAs bind to ribosomes more rapidly and to a greater extent than their uncapped counterparts.

This important observation has raised the additional question of how

those mRNAs that are not normally capped function as templates for protein synthesis; in particular, how do bacterial messengers cope with this problem and what would be the effects of introducing caps on to these molecules? Some of the answers to these questions are supplied by two papers by Paterson and Rosenberg in this issue of *Nature* (page 692). They have found that several prokaryotic mRNAs can be translated quite efficiently in a cell-free system derived from wheat germ provided that the 5' ends of these messengers are modified by addition of a methylated cap structure. This modification was achieved in two ways, either using the endogenous capping activity of the wheat germ system with S-adenosyl methionine as methyl donor, or using enzymes purified from vaccinia virus to add the cap and then re-extracting the RNA before adding it to the cell-free system. By such manipulations the efficiency of translation of defined mRNAs of bacteriophage  $\lambda$  and of the *E. coli* gal mRNA could be made comparable with that of the eukaryotic globin mRNA.

These results must indicate considerable evolutionary conservation of the mechanism by which the protein synthetic machinery recognises its templates; apart from the absence of a cap the normal structural features of bacterial mRNAs appear to be sufficient to permit their utilisation by the wheat-germ extract. But how do bacterial ribosomes bind to uncapped bacterial mRNAs? Clearly there must be different structural features of the latter which facilitate such interactions. One such feature is the presence of nucleotides on the 5' side of the initiating AUG codon which can form base pairs with sequences at the 3' end of 16S ribosomal RNA (Shine and Dalgarno *Proc. natn. Acad. Sci. U.S.A.* **71**, 1342; 1974). It is interesting that eukaryotic mRNAs, in contrast, generally cannot form extensively base-paired complexes with eukaryotic 18S rRNA and, as pointed out by Rosenberg and Paterson, neither can

the mRNAs they have used. One reason for the appearance of cap structures in higher cells may therefore be to replace the direct mRNA-rRNA interaction which facilitates ribosome binding in prokaryotic organisms. Because cap structures appear not to bind to ribosomes directly, but require one or more additional protein initiation factors for this process, we may also have a partial explanation for the greater number of such factors needed for successful eukaryotic protein synthesis, relative to the prokaryotic process.

We are now in a position to see in outline how eukaryotic cell ribosomes may find the AUG initiation codons on mRNAs, according to the model recently proposed by Kozak (*Cell* **15**, 1109; 1978). Native small ribosomal subunits carrying initiator methionyl-tRNA bind at or near the 5' cap structure and then move along the RNA, scanning its sequence until an AUG is soon encountered, which will be complementary to the anti-codon on the tRNA. The large ribosomal subunit then attaches and protein synthesis can commence. Thus, polypeptide assembly begins at the first AUG codon occurring at the 5' end of the RNA. A prediction from this model is that internal AUG triplets are not used as initiation sites and that eukaryotic ribosomes cannot translate cistrons other than the 5'-proximal one in polycistronic mRNAs, even after cap addition. This prediction is borne out by the results described by Rosenberg and Paterson, making it tempting to suggest that cap-dependent initiation is the reason for the absence of functional polycistronic mRNAs from higher cells.

In addition to the mechanistic implications of these experiments, some practical considerations arise concerning the efficient expression of foreign

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genes in both animal and bacterial cells. The addition to prokaryotic mRNAs of cap structures, with or without the non-coding sequences adjacent to them, by means of chemical, enzymatic or genetic engineering

techniques, could amplify the synthesis of certain bacterial gene products in higher cells (assuming the RNA can be introduced successfully into the cells). Conversely, there may not be a need for 5' caps on eukaryotic mRNAs

which are to be translated within bacterial cells. Other structural features of the molecules, including their potential ability to base pair with 16S rRNA and to be recognised by bacterial factors, may be more important. □

## Processing of protein precursors

from D. F. Steiner

WORK with a wide variety of secretory proteins has by now amply substantiated the 'signal hypothesis'. Proteins that are eventually going to be secreted are synthesised on ribosomes attached to the endoplasmic reticulum (ER) and transferred into the lumen of the ER from where they are finally released from the cell. The signal hypothesis states that these proteins initially contain an N-terminal 'signal' region which during translation guides the attachment of the ribosomes to the ER. The signal sequence is cleaved off shortly after the protein is segregated into the cisternae of the ER prior to secretion.\*

One difficult exception to the signal hypothesis has been ovalbumin, which is secreted into the ER and glycosylated without the apparent participation of a cleaved signal sequence. However, the laboratories of both R. Palmiter (University of Washington, Seattle) and G. Blobel (*J. Cell. Biol.* **79**, 567; 1978) have found that ovalbumin competes with other presecretory proteins for transfer into microsomes in reconstituted translational systems, implying the presence of a functional leader sequence. This apparent paradox has now been resolved by a dramatic announcement by V. Lingappa *et al.* from Blobel's group (Rockefeller University) that a prepeptide-like 'signal equivalent' sequence can be released from ovalbumin by tryptic digestion and that this sequence is located more than 200 residues into the molecule. This region contains all the competing activity, and from its position and composition can be inferred to contain a modified version of the typical N-terminal presequences of several other oviduct presecretory proteins (studied by Palmiter's group). It is not yet known whether this region functions as a signal peptide in the transfer of ovalbumin across the ER membrane,

and if it does, how the very long N-terminal region of the nascent chain could be transferred.

However, the finding of an apparent signal sequence at an internal site rather than at the N-terminal end itself, lends support to the 'loop' models of protein transfer into the ER, as against the membrane pore model, originally put forward by Blobel and Dobberstein, in which it was postulated that proteins are threaded through membranes, N-termini first. The loop models proposed by Inouye (*A. Rev. Biochem.* **47**, 481; 1978) and by Steiner *et al.* (*Proc. int. Symp. Proinsulin, Insulin and C-peptide*, 1978, Elsevier, in the press) suggest that the lipophilic signal sequence interacts with the membrane and assumes an inverted transmembrane orientation. Steiner and colleagues at the meeting suggested that the 9-10-residue region of the signal sequences interacts with components spanning the membrane and that successive formation of hydrogen bonds within the apolar membrane core between the pre-existing and the entering prepeptide strands produces a peptide loop across the membrane. Such a receptor mechanism could depend more on secondary than on primary structure and could explain the apparent lack of specificity in the binding of the various presequences. The loop mechanism is supported by preliminary evidence from P. Quinn *et al.* (University of Chicago) that the N-terminal region of the lead peptides of rat growth hormone and human placental lactogen (HPL) remain outside vesicles in *in vitro* transfer experiments and are released into the medium as small residual peptides.

As G. Scheele (Rockefeller University) pointed out in discussion, the loop model may provide a mechanism for incorporating multiply inserted proteins into membranes. It also seems possible that it might provide a means for inserting transmembrane proteins with 'inverted' N → C polarity across the membrane.

### Converting enzymes

Protein processing is not confined to the cleavage of signal sequences, however. Most polypeptide hormones, for example, are synthesised as much longer 'prohormones' which are subsequently processed to produce the biologically active product. The nature of the trypsin-like and carboxypeptidase B-like enzymes responsible for the cleavage of the paired basic residues (which mark the cleavage sites) in many of the prohormones remains unclear. The cleavage of nerve growth factor and epidermal growth factor (discussed by E. Shooter, Stanford University) is carried out by specific trypsin-like peptidases which form one of the subunits of the high molecular weight complexes in which these growth factors occur. The complexes thus seem to resemble proteinase/inhibitor complexes.

The signal peptidase activity in detergent-treated microsomes has been partially characterised. There is now general agreement that it is a neutral endopeptidase latent within untreated microsomes and is probably associated with the luminal face of the microsomal membrane. Its inhibition by high concentrations of 1,10-phenanthroline and several thermolysin inhibitors (M. Zimmerman *et al.* Merck, Sharpe and Dohme, Rahway, New Jersey) suggest that it may be a metalloprotease.

An interesting viral cleavage system is that of Sindbis virus. In this case a multicistronic mRNA encodes the capsid protein followed, without punctuation, by the two virus glycoproteins which become membrane-associated. Schlesinger *et al.* (Washington University, St Louis) have shown that the nascent polypeptide itself cleaves off the N-terminal capsid portion autocatalytically before tandem translation of the glycoproteins which are then vectorially coupled to fatty acids within the membrane and cleaved

\*A conference on Precursor Processing in the Biosynthesis of Proteins was held in New York City on 2-4 May, sponsored by the New York Academy of Sciences. It was organised by D. F. Steiner and M. Zimmerman.

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to their mature peptide forms. The tandem arrangement of these proteins suggests again the possibility that a functional signal sequence need not be at the N-terminus of a nascent polypeptide.

### Genetics

The advent of recombinant DNA techniques has greatly facilitated studies of the structure of the genes coding for protein precursors. Most remarkable at the meeting was the wealth of information gained from structural studies at the nucleotide level by Howard Goodman and coworkers on cloned mRNAs for rat and human growth hormones and the related peptide human chorionic sommatomammotropin (HCS). Studies of the corresponding chromosomal genes of HGH and HCS indicate the presence of multiple intervening sequences, while the gene for rat insulin I from the hooded rat was found to contain only a short intervening sequence in the 5' untranslated portion of the gene. Further studies on cloned rat preproinsulin genes derived from a Wistar-related strain, reported by P. Lomedico *et al.* from W. Gilbert's laboratory (Harvard University) confirmed the presence of a single intron (of 119 bp) upstream from the initiation codon in the gene for rat preproinsulin I, while the gene coding for preproinsulin II contained an additional intron of about 500 nucleotides between the region encoding amino acids 38 and 39 of the proinsulin II peptide chain (thus being in the C-peptide region). Their finding of nuclear RNA molecules larger than preproinsulin mRNA, confirming an earlier report by Duguid *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **75**, 3249; 1978) indicates that the intervening sequences are transcribed into precursor mRNAs. Similarly in studies on growth hormone biosynthesis in rat pituitary GH cells pulse-labelled with uridine F. C. Bancroft *et al.* (Sloan-Kettering Institute, New York) have identified a nuclear 20S mRNA precursor which is slowly processed to the mature 12S mRNA of the hormone.

N-terminal partial sequence studies of the pro-opiocortin precursor of the cell-free translation product of its mRNA by E. Herbert *et al.* (University of Oregon, Eugene), and by N. G. Seideh *et al.* from M. Chretien's laboratory (McGill University), have confirmed the presence of a signal sequence predicted from the recently completed nucleotide sequence of the DNA (Nakinishi *et al.* *Nature* **278**, 423; 1979). They have also established the presence of a cleavage site between Gly-Trp, 26 residues in from the initiator codon. R. Mains and B. Eipper (University of Colorado, Denver) presented recent studies showing that one of the pro-opiocortin precursors (31K) is processed

differently in pars intermedia than in the anterior pituitary, favouring production of  $\alpha$  melanocyte stimulating hormone in the former and of adrenocorticotrophic hormone in the latter tissue. The basis for this difference in processing in the two tissues is unknown.

An exciting new approach to studies at the nucleotide level of less abundant mRNA species came from the demonstration by K. Agarwal *et al.* (University of Chicago) that synthetic nucleotide primers can be utilised to detect and selectively transcribe into cDNA those mRNAs which are present below the 1% level in poly A-containing RNA fractions from tissues. Application of this approach to studies on gastrin mRNA mixtures led to the successful identification of a 620-nucleotide mRNA coding for gastrin G34 and indicated the presence of additional amino acid sequences on both sides of the G34 coding region (*Proc. natn. Acad. Sci. U.S.A.* **76**, 1770; 1979). These results predict that progastrin is 110–140 amino acids long and provide the necessary means to purify and ultimately clone the mRNA for this hitherto unexplored precursor molecule. This method is obviously of wider applicability and will undoubtedly provide a new strategy for identifying and amplifying important gene and mRNA structures.  $\square$

## Is unification really true?

from Norman Dombey

THERE is a general consensus in the world of elementary particle physics that electromagnetic interactions and weak interactions (responsible for nuclear  $\beta$ -decay) are just different aspects of the same theory (*News and Views* **278**, 209; 1979). The predictions of the standard unified gauge theory of weak and electromagnetic interactions based on the group  $SU(2) \times U(1)$  have been verified in every high energy scattering experiment so far performed, culminating in the remarkable demonstration of parity violation at Stanford last year when a difference was observed in the scattering of left-handed electrons and right-handed electrons off a deuterium target (*News and Views* **274**, 11; 1978). Indeed the July/August issue of the *CERN Courier* then remarked that the only remaining problem in the field was to find a suitable name for the unified theory.

A note of caution was sounded recently by Barut (*Nature* **278**, 692; 1979) who noted that the crucial test for a unified theory was the observation of the spectrum of gauge particles or quanta of the theory

with masses of the scale  $e/G^{\frac{1}{2}} \sim 90$  GeV where  $e$  is the electric charge and  $G$  is the Fermi constant of the weak interactions. The combination of the two basic coupling constants in this way would be the decisive feature of a unified theory.

I shall attempt here to take this comment further and to review critically the present evidence for unification. I will indicate in fact that the successes of the standard theory could be the result of the inclusion in that theory of assumptions which have nothing to do with the unification of weak and electromagnetic interactions.

The experiment situation as far as high energy scattering is concerned can be summarised thus: all neutrino neutral current experiments with nucleon targets whether inclusive (that is of the form  $\nu N \rightarrow \nu X$  where  $X$  is unobserved) or exclusive (for example,  $\nu N \rightarrow \nu N$ ,  $\nu N \rightarrow \nu N \pi$ ) together with the Stanford polarised electron experiment are consistent with a neutral weak current of the form written down by Glashow (*Nucl. Phys.* **22**, 579) in 1961 in his pioneering attempt to unify weak and electromagnetic interactions through the group  $SU(2) \times U(1)$ . This current is

$$J_{\mu}^w = V_{\mu}^3 + A_{\mu}^3 - 2\sin^2\theta J_{\mu}^{\text{em}} \quad (1)$$

where  $V_{\mu}^3$  and  $A_{\mu}^3$  are the neutral isospin components of the vector and axial currents whose charged components constitute the current of the normal  $V-A$  theory of  $\beta$ -decay, and  $J_{\mu}^{\text{em}}$  is the electromagnetic current. Experimentally  $\sin^2\theta = 0.24 \pm 0.02$  (*Proceedings of Neutrino 78*, 253, Purdue University Press, 1979).

Glashow, however, was unable to predict the strength of neutral current scattering by neutrinos compared with charged current scattering (that is  $\bar{\nu} + N \rightarrow \mu^-(\mu^+) + X$ ) because he did not know the relation between the mass  $M_Z$  of the neutral gauge particle  $Z^0$  of his theory compared with the mass  $M_W$  of the charged gauge particles  $W^{\pm}$ . The strength of charged current scattering is given by the Fermi constant  $G$  where  $G/\sqrt{2} = g^2/8M_W^2$  as a  $W^+$  or  $W^-$  is exchanged in the process ( $g = e\sin\theta$  is the weak coupling constant). For scattering by neutral currents the appropriate Fermi constant  $G'$  is given in  $SU(2) \times U(1)$  by  $G'/\sqrt{2} = g^2/16M_Z^2\cos^2\theta$  as now a  $Z^0$  is exchanged.

This problem was solved by Weinberg (*Phys. Rev. Lett.* **19**, 1264) in 1967 by using the Higgs mechanism of spontaneous symmetry breaking to generate the masses  $M_W$ ,  $M_Z$  thereby finding that  $M_W = M_Z\cos\theta$ . Hence the relation between  $G'$  and  $G$  in the standard theory is just

$$G' = \frac{1}{2}G \quad (2)$$

The best value for the neutral current

strength in neutrino scattering is conveniently written in terms of  $\kappa = M_Z \cos \theta / M_W$ . Then  $\kappa^2 = 0.98 \pm 0.05$  (*Proc. Neutrino 78*, 253) in close agreement with the Weinberg value  $\kappa = 1$ .

Thus the standard theory predicts with remarkable accuracy both the form and the strength of the weak neutral current in high energy scattering experiments. The only difficulty at present in the experimental verification of the theory lies in the conflicting results on atomic parity violation in bismuth (*Proc. Neutrino 78*, 417; *News and Views* 264, 505; 1976) and thallium (*Phys. Rev. Lett.* 42, 343; 1979): the Washington experiment which has the smallest reported errors disagrees by some seven standard deviations from the predictions of the standard theory. These experiments, however, are very difficult and require for their interpretation a model of heavy atoms of uncertain validity.

Hence the general consensus that the standard theory has been verified. The sceptic must now remark that all the data so far observed are consistent with the old Fermi four-fermion theory of weak interactions, provided that neutral currents are included with the form (1) and strength (2). No additional structure in weak interactions due to the existence of gauge particles  $W^\pm$ ,  $Z^0$  with their prescribed masses of 78 and 90 GeV respectively has been observed, nor is there any experimental evidence yet for the scalar Higgs particle required by the theory. Neither (1) or (2) is especially complicated so there may well be a simple explanation of these two relations which does not require the untested features of the standard unified theory: in particular the predicted masses for  $W^\pm$  and  $Z^0$  and the existence of the Higgs scalar.

Hung and Sakurai (*Nucl. Phys.* B143, 81; 1978) did indeed find recently an extremely simple explanation of (1) and (2). They showed that starting with the isospin- or SU(2)-conserving V-A theory of weak interactions including neutral currents due to Bludman (*Nuovo Cimento* 9, 433) in 1958, then the  $W^0$  which couples to the neutral weak current and the photon  $\gamma$  which couples to the electric current must mix quantum mechanically. This is because both electrons and quarks can be left-handed (for the V-A weak coupling) and charged. If this calculation is done carefully, making sure that the photon is massless both before and after mixing, then not only is the Glashow current (1) obtained with  $\sin^2 \theta$  as a measure of the mixing—which it is in the standard theory anyway—but also the mixing does not change the relation  $G' = \frac{1}{2}G$  which comes simply from isospin invariance in

the underlying theory. Thus the two successful predictions of the standard theory are obtained from a mixing calculation with no extra assumptions about unification, gauge theories or Higgs particles. From this point of view all the successes of the standard unified gauge theory are just due to the correct incorporation of  $\gamma$ - $W^0$  mixing in the formalism.

Hung and Sakurai consider that their calculation is an alternative approach to weak and electromagnetic interactions that contains the standard theory and may but does not necessarily, give different results. They emphasise that from the mixing point of view,  $\sin^2 \theta$  should be

considered as a function of momentum transfer  $q$  and not as a fundamental constant, and that the electron and muon neutrinos would have different interactions with the electromagnetic field, and therefore different values of  $\sin^2 \theta$  should be associated with different neutrinos. Their motivation seems to be mainly a dislike of the Higgs particle which is a necessary ingredient of the standard theory but whose mass is arbitrary and is not predicted by the theory. The number of Higgs particles is also arbitrary but in the standard theory, the simplest choice of one Higgs is made, thereby obtaining relation (2).

Although Hung and Sakurai do not



## A hundred years ago

### SOCIETIES AND ACADEMIES

BOSTON (U.S.A.)

**American Academy of Arts and Sciences**, May 14.—Hon. Charles Francis Adams in the chair.—Dr. H. P. Bowditch presented a new form of plethysmograph differing from those of Mosso and van Basch in the method adopted for securing a constant level of the fluid in the receptacle connected with the apparatus which contained the body whose changing volume was to be measured. The method consisted in suspending the receptacle (a large sized test tube) to a delicate spiral steel spring of which the length and strength were so adjusted that the weight of the fluid flowing into the test tube caused an elongation of the spring precisely equal to the rise of the fluid in the test tube itself. Thus the absolute level of the fluid in the receptacle remained unaltered, and a constant pressure was maintained upon the surface of the organ to be measured. An index attached to the lower end of the spring recorded upon a revolving cylinder covered with smoked paper the flow of the fluid into and out of the receptacle.—Mr. N. D. C. Hodges gave two new proofs of the dimensions of molecules, one based upon the properties of water and aqueous vapour, the other upon superficial tension and considerations of the depth of the superficial layer of molecules upon sheets of platinum.—Prof. Pickering exhibited a new form of photometer for measuring the light of a nebula or comet, by comparison with a star thrown out of focus. The method employed eliminated the effects of moonlight or twilight. He also proposed to denote the light of these bodies in stellar magnitudes. Thus a portion of a nebula would be of the twelfth magnitude, if of the same brightness as a twelfth-magnitude star spread over a circle one minute in diameter.

### Evolution Old and New

MR. A. R. WALLACE writes, in *NATURE*, vol. xx. p. 143, that, according to the theory which I support, Australian (and more especially Queensland) sheep should show a tendency to grow a scantier and thinner fleece than their English ancestors. "If Mr. Butler," he continues, "could adduce on good authority such a fact as this, he would have some evidence in his favour, instead of which he can only make suppositions."

I never was in Australia, but had some years' experience of sheep-farming in New Zealand. It was generally believed, in my time, that fleeces soon became short and hairy in Queensland, and even in the more northern part of New South Wales. You must, however, have many readers who could tell us what the facts are. May I hope that you will kindly insert this, so as to get the matter settled by eliciting information from a competent authority? I am speaking, of course, of sheep that are left to the effect of the climate, without being frequently crossed with rams from colder countries. Do the fleeces of such sheep deteriorate in Queensland? S. BUTLER

June 12

### Oxygenated Rain

ON Thursday, June 12, at half-past eleven in the morning, a remarkable shower of rain fell over London, which might almost be described as "effervescent;" the drops whilst falling appeared to be colourless and perfectly transparent, but on striking against any solid surface they became milky, and on close examination it was evident that this cloudy appearance was caused by a number of very minute air-bubbles, which rapidly increased in size, and then burst. From the bleaching power which this rain appeared to have, I am led to believe that there was nascent oxygen in the gas thus evolved. Those who traverse the streets of London in the early morning may now and then observe the red colour of all bright iron-work in the pavement, such as coal-plates, &c., due to the oxidising influence of a thunder-shower in the night; this effect does not follow every thunder-shower, but seems to indicate a peculiar atmospheric condition. Have any memoranda on this subject been recorded? EDWARD SOLLY

From *Nature* 20, 19 June, 169, 188; 1879.

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claim that a theory different to the standard theory results from their work, it is possible to envisage a radical alternative to the standard theory starting from  $\gamma$ - $W^0$  mixing. The motivation now is that if  $\sin^2\theta$  were essentially a radiative correction as these ideas suggest then  $\sin^2\theta \approx \alpha \approx 1/137$  whereas experimentally  $\sin^2\theta \approx 1/4$ . This means that the radiative corrections must be much larger than expected; yet they can only be much larger if higher order weak interactions are important. This implies that  $g^2/4\pi \gtrsim 1$  whereas in the unified theory  $g = e\sin\theta$  so  $g^2/4\pi < \alpha$ . Hence now  $M_W, M_Z \gtrsim 1/G^1 = 300$  GeV.

Of course the alternative approach is not a complete theory, it is just a calculation of quantum mixing which must be part of any theory. So if the radical alternative is taken seriously, it is necessary at some stage to consider what happens at high energies ( $\gtrsim 300$  GeV) where the four-fermion theory must break down. What does happen cannot yet be predicted but significant progress has been made recently on treating the four-fermion interaction as a serious theory in its own right by Cooper, Guralnik and their collaborators (*Ann. Phys.* **109**, 165; 1977; *Phys. Rev. Lett.* **40**, 1620; 1978; *Phys. Rev.* **D19**, 549, 562; 1979). The ideas here are based on the theory of superconductivity which of course also involves a four-fermion interaction, albeit one which is non-relativistic. The calculations are not performed perturbatively but in a mean field approximation as in many-body theory. Both vector (gauge) and scalar (Higgs) particles now appear as dynamical excitations of the theory, not as elementary entities, thereby removing the arbitrariness of the Higgs particle mass which many see as the most serious failing of the standard theory.

The theory of superconductivity may well provide a good analogy for weak interaction theory. Englert (International Centre for Theoretical Physics, Trieste, Report IC/76/92; 1976) has pointed out that the Higgs phenomenon has already been discovered experimentally, not in particle physics but in the Meissner effect. The range of penetration of the magnetic field is given by the photon mass induced by spontaneous symmetry breaking of the Ginsburg-Landau Lagrangian through the Higgs mechanism. Here the Higgs scalar can easily be understood: it is just the bound state Cooper pairing of the electrons of the BCS theory. The Ginsburg-Landau approach to superconductivity in terms of vector and scalar order parameters is the phenomenological theory, whereas the basic physics lies in the BCS four-fermion theory. In the same spirit, the standard unified theory could be regarded as a phenomenological theory of weak interactions valid in the region  $q^2 \ll M_W^2$ , or perhaps,  $m_e^2 \ll q^2 \ll M_W^2$  ( $m_e$  is the electron mass), while the four-fermion theory is funda-

mental. In a Los Alamos Report (LA-UR-79-306; 1979) I have noted that the latter possibility would allow  $\sin^2\theta \approx 0$  in atomic physics where  $q^2 \lesssim m_e^2$  thereby giving a value for atomic parity violation in bismuth or thallium about three times smaller than in the standard theory, a result which agrees with all the experiments performed to within 2.5 standard deviations.

If the radical alternative is correct, then two final remarks are appropriate. The first is theoretical. Weak interactions now become strong at energies of 300 GeV, they do not level off at a typical electromagnetic strength at 90 GeV or so. This provides a physical reason for a grand unification of leptons and hadrons at high energies which is missing from the usual viewpoint. The second remark is experimental. No  $Z^0$  will be seen at LEP for 70 GeV electrons on 70 GeV positrons. To ensure seeing some effects of weak interaction structure a centre of mass energy of at least 300 GeV is needed.  $\square$

## Oscillations in cellular reactions

by Eleanor Lawrence

A RECENT meeting\* in the Black Forest brought together some of those working in the field of cellular oscillating reactions. The periodic rise and fall in concentrations of metabolites or in membrane potential is found in a wide variety of cells. In some cases, such as the membrane potential changes in cardiac pacemaker cells, the physiological significance of the oscillating reaction is clear; in others the oscillatory phenomena may be a consequence of the underlying physico-chemical properties of the components of a particular reaction but may have no further physiological role. However, the general point was made at the meeting that an oscillating reaction provides a stable and energy-efficient signal whose modulation by metabolites, hormones or synaptic input could convey information rapidly and precisely to the cell itself or to its neighbours.

Some order, however arbitrary it

may eventually appear, can be imposed on this collection of diverse and seemingly unrelated phenomena by a division into 'cytoplasmic' oscillators on the one hand, of which the glycolytic oscillator is a prime example, and 'membrane' oscillators on the other. Self-contained models for oscillating membrane potential based on a cycle of inward and outward ionic currents are consistent with much of the experimental evidence. But whether membrane oscillators are in practice completely self-contained or whether they are in some cases driven by underlying internal oscillators is still an open question, and various possibilities were canvassed at the meeting.

### Glycolytic oscillator

Undoubtedly the cellular oscillator best characterised at the molecular level is the so-called glycolytic oscillator described by B. Hess (Max-Planck-Institut für Ernährungsphysiologie, Dortmund). Periodic regular oscillations in the concentrations of glycolytic intermediates were discovered first in yeast cells and later in extracts of skeletal muscle and heart. The key component producing the oscillations in this pathway is the allosteric enzyme phosphofructokinase (PFK), which catalyses the phosphorylation of fructose-6-phosphate into fructose-1,6-diphosphate using ATP as an energy source. The oscillations reflect the periodic changes in the activity of PFK (within a certain range of substrate input) in response to its activators F6P, ADP and AMP, and to its allosteric inhibitor ATP, which produces regular pulses of product to which the rest of the pathway is geared. The stability of the oscillations to the stochastic substrate input, expected normally, has been shown by experiments in which input was varied at random. In these conditions the reaction still oscillated at its 'normal' periodicity. Such oscillating reactions have the fascinating property of generating structure in initially homogeneous solutions, as Hess illustrated. When the reaction in a shallow unstirred yeast extract is followed by ultraviolet photography, which picks up the fluctuations in oxidised and reduced pyrimidine nucleotides, the solution soon takes on a regularly pulsing structure composed of discrete domains.

So far, however, evidence that the glycolytic oscillator could provide a basis for other oscillatory phenomena is sparse. R. Chaplain (University of Mainz) is accumulating evidence suggesting a link between glycolysis and the membrane potential oscillations represented by the periodic autonomous firing of certain neurosecretory neurones in the abdominal ganglion of the sea-hare *Aplysia*. I. Levitan and his

\*A meeting on 'Cellular Oscillators', organised by M. J. Berridge and P. E. Rapp, and sponsored by the Company of Biologists and Dr Karl Thomae GmbH, was held at Titisee on 22-24 March 1979. The proceedings will be published as Volume 81 of the *Journal of Experimental Biology* due out in August 1979.

colleagues (Friedrich-Miescher-Institut, Basel) with a different neurone in the same ganglion, find that oscillatory behaviour can be profoundly changed by changes in the internal concentrations of cyclic nucleotides. The inhibitory effects of synaptic stimulation can be mimicked by increases in cyclic AMP alone. The enhanced activity induced by vasopressin and oxytocin is simulated by increases in cyclic GMP and cyclic AMP together. These hormones naturally cause a rise in cyclic nucleotide levels. Levitan has also identified hormonal activity resembling that of vasopressin and oxytocin in peptide-containing extracts of molluscan ganglia.

#### Membrane oscillators

Detailed dissection of the ionic mechanisms of membrane potential oscillations reveals a slightly different picture in different tissues. However, in all cases the oscillatory behaviour appears to be due primarily to a slow outward  $K^+$  current which is normally responsible for membrane hyperpolarisation. As this current gradually inactivates it initiates membrane depolarisation leading into the upswing of the next action potential.

In cardiac pacemakers (the frog sinus venosus and the mammalian sinoatrial node) described by H. Brown and S. Noble (University of Oxford) and the Purkinje fibres described by R. Tsien (Yale University School of Medicine) the dominant pacemaking oscillation in membrane potential can be explained by a self-contained cycle of inward and outward currents which are activated and inactivated by the changes in voltage they produce. Brown and Noble together with D. DiFrancesco have been looking at the mechanism of the inhibition of pacemaking in the heart by acetylcholine and its acceleration by adrenaline. They find that acetylcholine in both mammals and amphibia greatly increases outward potassium current, possibly by opening up a special ACh-activated potassium channel. Adrenaline on the other hand, increases the slow inward  $Na^+/Ca^{2+}$  current and also affects a newly discovered additional pacemaking current, overriding the adrenaline-induced increase in outward current.

Although the dominant pacemaking membrane oscillations can be explained by a self-contained membrane model, as Tsien pointed out, pacemaker cells also probably contain a separate 'internal' oscillator largely independent of the main membrane oscillator, but which reveals itself in oscillatory changes in membrane conductance and cell contractility even when the mem-

brane potential is fixed. This internal oscillator is enhanced in conditions that raise internal free calcium, leading Tsien to suggest that the membrane conductance oscillations are driven by a cycle of uptake and release of Ca from some intracellular store, probably the sarcoplasmic reticulum. Increased intercellular free calcium is known to increase membrane permeability to  $K^+$ .

A cycle of Ca uptake and release, this time by the endoplasmic reticulum is invoked by P. G. Nelson (National Institutes of Health) to explain the oscillations in membrane potential seen in L cells subjected to mechanical or electrical stimuli. On electron microscopic evidence of specialised structures closely apposed to the membrane, Nelson proposes a model in which Ca-induced changes in membrane permeability to  $K^+$  are coupled to a cycle of Ca uptake and release from the ER. A similar  $Ca^{2+}$  cycle might also drive the periodic contractions in the veins of the acellular slime-mould *Physarum* (described by K. E. Wohlfarth-Bottermann, University of Bonn).

The role of calcium in controlling the frequency of bursts in the molluscan burster neurones was emphasised by R. W. Meech (ARC Unit of Invertebrate Chemistry and Physiology, Cambridge). He suggests that the prolonged inward current carried by  $Na^+$  and  $Ca^{2+}$ , which is responsible for the depolarising phase of the burst, is also responsible for a rise in intracellular Ca which then triggers the slowly developing outward  $K^+$  current responsible for the hyperpolarising interburst phase.

#### Link with glycolysis

A link with both glycolysis and cell Ca concentration is proposed by E. K. Matthews (University of Cambridge) for the membrane oscillations elicited in pancreatic islet cells stimulated to release insulin by glucose. The membrane oscillations themselves are again driven by changes in  $K^+$  conductance but in this case Matthews proposes a link with glycolysis. Glucose metabolism leads to a decreased permeability to  $K^+$  and so to depolarisation. The principal ion entering the cell during the bursts of action potentials superimposed on the crests of the membrane oscillation, is thought to be  $Ca^{2+}$ , which could also provide negative feedback, increasing  $K^+$  permeability and initiating the hyperpolarising interburst phase.

One organ in which the physiological role of cellular oscillations is clear although their mechanism is less clear, is the intestine. The smooth muscle in the wall of the stomach and intestine displays fairly slow oscillations in membrane potential—the 'slow wave'—which propagates along the intestine

(J. A. Connor, University of Illinois, Urbana). Contraction is initiated when action potentials are generated on the crest of the wave. In the cat at least, pacemaker activity seems to reside in the longitudinal layer of the musculature. Preliminary attempts to identify the primary oscillator point, in Connor's opinion, to rhythmic modulation of current from an electrogenic sodium-potassium pump, although other metabolic processes may be involved.

#### Chemical signals

Returning to 'metabolic' oscillators, one of the most spectacular is the aggregation of the amoebae of the cellular slime-mould *Dictyostelium* as a result of the propagation and amplification from cell to cell of a chemotactic signal carried by cyclic AMP. G. Gerisch (Biozentrum, Basel) speculated on possible metabolic control pathways leading to the periodic synthesis of cyclic AMP pulses. On the basis of strong evidence of oscillations in cyclic GMP activity, Gerisch has proposed a tentative scheme of feedback control loops in which cyclic AMP binding to the external receptor leads to an immediate rise in cyclic GMP which then activates adenylyl cyclase leading to increased cyclic AMP production. This in turn damps down the receptor-mediated increase in cyclic GMP. Gerisch sees the oscillations in adenylyl cyclase activity (possibly mediated by phosphorylation of the enzyme) as the primary oscillation in the pathway in contrast to other models which have taken the allosteric change in the receptor on binding cyclic AMP as the key component.

M. Israel (CNRS, Gif-sur-Yvette) described oscillations in the intracellular concentrations of ACh in *Torpedo* electroplax during stimulation. On the apparent fairly long-period rise and fall in intracellular ACh are imposed very short-period changes in ACh concentration which oscillate in phase with ATP concentration. As the amplitude of electrical discharge (reflecting transmitter release) does not oscillate, they conclude that transmitter output is not directly related to ACh concentration.

#### No unification

Summing up, M. Berridge (ARC Unit of Invertebrate Chemistry and Physiology, Cambridge) pointed the moral of the meeting—that there is no universal oscillator underlying the different phenomena described. Although the influence of calcium pervades many discussions of oscillatory mechanisms and Rapp and Berridge (*J. theor. Biol.* **66**, 497; 1977) have proposed the attractive notion that control loops involving calcium and cyclic nucleotides may be involved in many oscillating reactions, the existence of such controls remains to be demonstrated experimentally, and they are unlikely to be universal. □

# review article

## Coated pits, coated vesicles, and receptor-mediated endocytosis

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*Proteins and peptides can enter cells by receptor-mediated endocytosis, a coupled process by which selected extracellular proteins or peptides are first bound to specific cell surface receptors and then rapidly internalised by the cell. Internalisation follows clustering of the receptors in specialised regions of the cell surface called coated pits that invaginate to form intracellular coated vesicles. It is now recognised that receptor-mediated endocytosis has a fundamental role in the growth, nutrition and differentiation of animal cells.*

CERTAIN proteins that bind to receptors on the surfaces of animal cells are rapidly internalised by the cells before they can dissociate from their receptors. This process, which is called receptor-mediated endocytosis, has recently become recognised as an important and general mechanism by which animal cells take up nutritional and regulatory proteins from extracellular fluid. Biologically important molecules known to be taken up by this mechanism include plasma transport proteins (such as low density lipoprotein and transcobalamin II), certain polypeptide hormones (such as insulin and epidermal growth factor), asialoglycoproteins, and lysosomal enzymes. In many cases, rapid internalisation of receptor-bound proteins is achieved by the clustering of receptors in specialised regions of the surface membrane called coated pits that invaginate rapidly into the cell during endocytosis to form coated vesicles<sup>1,2</sup>. Coated pits and coated vesicles have recently acquired the status of subcellular organelles because they can be separated from other cellular components and because they possess a characteristic structure and protein composition<sup>3,4</sup>.

We review here some of the experiments that have delineated the process of receptor-mediated endocytosis. We rely heavily on data gathered from extensive study of the low density lipoprotein (LDL) receptor system, which functions to transport cholesterol from plasma into cells<sup>5,6</sup>. We then review 12 other protein transport systems for which receptor-endocytosis has been documented. From such data, we deduce certain general features of the process that may apply in most types of animal cells.

### Receptor-mediated endocytosis as a mechanism for cellular protein uptake

Endocytosis is a general term that refers to the process by which cells ingest extracellular materials by trapping them within inward foldings of the plasma membrane that pinch off from the surface to form intracellular vesicles<sup>7</sup>. Endocytosis occurs widely in protozoa where it has an obvious nutritional role. In

multicellular organisms endocytosis was first identified in macrophages and other phagocytic cells that ingest foreign particulate materials and deliver them to lysosomes for degradation. With the advent of the electron microscope, it was recognised that endocytosis is not limited to phagocytic cells but occurs in all animal cells<sup>8</sup>. The rate of endocytosis in non-phagocytic cells can be appreciable. For example, mouse L cell fibroblasts internalise an amount of cell surface membrane equal to 50% of the cell surface every hour<sup>7</sup>. One reason for such rapid endocytosis now seems clear: it provides a mechanism by which cells take up necessary macromolecules from extracellular fluid in controlled conditions.

Within the past few years, at least 13 systems have been described in which non-phagocytic cells use endocytosis to internalise proteins that have become bound to surface receptors. These systems share four properties that collectively define receptor-mediated endocytosis: (1) The binding component on the cell surface is a receptor in the strict sense, that is, it is a molecule whose function is to bind an endogenous ligand and thereby achieve a physiological effect. This criterion distinguishes these receptors from other cell surface components that serve as binding sites for opportunistic foreign molecules, such as lectins and toxins, and viruses. (2) Internalisation of the ligand is effectively coupled to binding. Once the protein is bound to the receptor, the half-time for internalisation is less than 10 min<sup>9-18</sup>. (3) In all cases for which ultrastructural data are available, the receptor-bound proteins enter the cell through coated pits, either because the receptors spontaneously localise in these regions or because binding of the ligand induces migration of the receptors to coated pits<sup>2,19-24</sup>. (4) The internalised proteins are usually delivered to lysosomes where they are degraded completely to amino acids<sup>2,6,12-18,25,26</sup>; occasionally processing of the internalised protein involves delivery to cellular structures other than lysosomes (see below).

The LDL receptor system illustrates each of the four characteristics of receptor-mediated endocytosis. The similarities and differences between the LDL receptor pathway and other systems of receptor-mediated endocytosis are presented in Table 1 and discussed below.

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**Table 1** Systems for receptor-mediated endocytosis of proteins

Protein	Cell type	Internalisation via coated pits and coated vesicles	Fate of internalised protein		Selected refs
			Degraded in lysosomes	Other	
<b>Transport proteins</b>					
LDL	Fibroblasts, smooth muscle cells, endothelial cells, adrenocortical cells, lymphocytes	Yes	Yes; cholesterol retained by cells	—	2, 6, 10, 52, 57
Yolk proteins (phosvitin, lipovitellin)	Oocytes (chicken, mosquito)	Yes	No	Delivered to yolk granules	21, 32
Transcobalamin II	Kidney cells, hepatocytes, fibroblasts	Data not available	Yes; vitamin B <sub>12</sub> retained by cells	—	14, 68, 69
Transferrin	Erythroblasts, reticulocytes	Yes	Data not available	Iron retained by cells	22, 60, 61
<b>Protein hormones</b>					
Epidermal growth factor	Fibroblasts, 3T3 cells	Yes	Yes	—	13, 23, 50
Nerve growth factor	Sympathetic ganglion cells	Data not available	Data not available	Carried in vesicles retro- grade up the axon	62
Insulin	Hepatocytes, hepatoma cells, lymphocytes, adipocytes, 3T3 cells	Data not available	Yes	Also delivered to Golgi apparatus and nuclei	12, 25, 50, 63, 64
Chorionic gonado- tropin	Leydig tumour cells, ovarian luteal cells	Data not available	Yes	—	15, 70
$\beta$ -Melanotropin	Melanoma cells	Data not available	Data not available	Delivered to Golgi apparatus and melanosomes	71, 72
<b>Other proteins</b>					
Asialoglycoproteins	Hepatocytes	Data not available	Yes	—	16, 17
Lysosomal enzymes	Fibroblasts	Data not available	No	Delivered to lysosomes and Golgi-associated structures; enzymes remain active for many days	65–67
$\alpha$ -2-Macroglobulin	Fibroblasts, macrophages, 3T3 cells	Yes	Yes	—	18, 24, 58
Maternal immunoglobulins (IgG)	Fetal yolk sac, neonatal intestinal epithelial cells	Yes	No	Transferred intact in coated vesicles to basal surface of cells where IgG is discharged into fetal or neonatal circula- tion	21, 47–49

## LDL receptor pathway as prototype for receptor-mediated endocytosis

Animal cells acquire cholesterol for synthesis of cell membranes by the receptor-mediated endocytosis of the cholesterol-carrying lipoprotein LDL, a large spherical particle (220 Å in diameter  $M_r \sim 3 \times 10^6$ ) that originates in the liver and circulates in the blood. Each LDL particle contains a nonpolar core composed of approximately 1,500 cholesterol molecules that are esterified to long-chain fatty acids. This cholesteryl ester core is surrounded by a polar coat of phospholipid, unesterified cholesterol, and protein<sup>6</sup>.

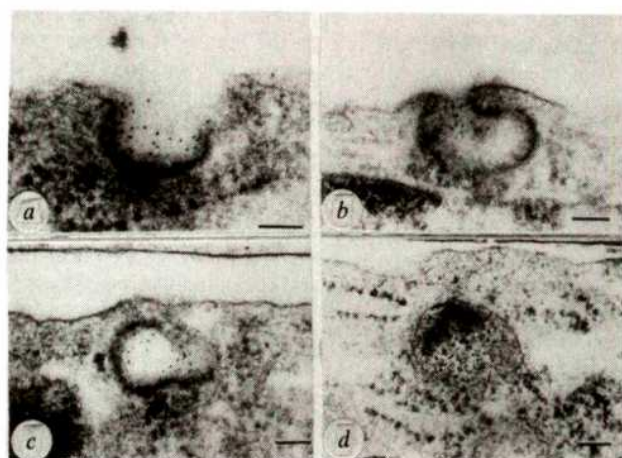
The receptor-mediated endocytosis of LDL was originally demonstrated in studies of human fibroblasts in tissue culture and has subsequently been found to operate in a wide variety of animal cells<sup>5,6</sup>. When cells require cholesterol for membrane synthesis, they synthesise a cell surface receptor that binds the protein component of LDL. Within 10 min of binding to the receptor, the LDL particle is internalised and delivered to lysosomes; in the lysosome the protein component is hydrolysed by proteases to amino acids and the cholesteryl esters are cleaved by an acid lipase to generate unesterified cholesterol. This unesterified cholesterol enters the cytoplasmic compartment where it serves two important functions: utilisation for membrane synthesis and regulation of key enzymes in cholesterol metabolism<sup>5,6</sup>. This latter action assures cholesterol

homeostasis in the cell as well as in the whole organism. Cytoplasmic cholesterol derived from the lysosomal hydrolysis of LDL also controls the synthesis of receptor molecules. Increased cellular cholesterol decreases the number of receptors, thereby preventing overaccumulation of LDL-cholesterol in the cell<sup>6</sup>.

The distribution of LDL receptors on the surface of fibroblasts has been studied by electron microscopy with the use of LDL coupled to the iron-containing, electron-dense protein ferritin<sup>2,19,20</sup> or by <sup>125</sup>I-LDL autoradiography<sup>27</sup>. Between 50% and 80% of all receptors are clustered over the 2% of the cell surface represented by coated pits (Fig. 1a). The membrane in these regions is indented and coated on its cytoplasmic surface by a bristle-coat. As LDL receptors are localised in coated pits even when the cells are fixed with formaldehyde prior to incubation with the LDL-ferritin, it is believed that receptor clustering occurs before the exposure to LDL<sup>19</sup>. Immunofluorescence studies with the light microscope show that the clusters of LDL receptors in human fibroblasts are aligned in linear arrays that appear to overlay actin-containing cellular stress fibres<sup>28</sup>.

LDL-ferritin bound to coated pits at 4 °C is rapidly internalised when the fibroblasts are warmed to 37 °C<sup>2</sup>. In this process the coated pits invaginate to form coated endocytic vesicles (Fig. 1a–c). After 5 to 10 min at 37 °C, LDL-ferritin is seen in lysosomes (Fig. 1d) as the result of their fusion with the





**Fig. 1** Electron micrographs showing the sequential binding of LDL-ferritin to coated pits, its internalisation in coated vesicles, and its delivery to lysosomes in human fibroblasts. Monolayers of growing human fibroblasts were incubated with growth medium containing 10% lipoprotein-deficient serum and LDL-ferritin at a concentration corresponding to  $47 \mu\text{g ml}^{-1}$  of LDL-protein at  $4^\circ\text{C}$  for 2 hours, after which the cells were washed extensively<sup>2</sup>. The cells then received growth medium containing 10% lipoprotein-deficient serum and were warmed to  $37^\circ\text{C}$ . After incubation at  $37^\circ\text{C}$  for the indicated time, the monolayers were fixed and embedded *in situ* and stained with uranyl acetate and lead citrate<sup>2</sup>. *a*, A coated pit that bound LDL-ferritin (time at  $37^\circ\text{C}$ : 1 min). *b*, A coated pit being transformed into a coated vesicle with LDL-ferritin included (time at  $37^\circ\text{C}$ : 1 min). *c*, A fully formed coated vesicle that seems to be losing its cytoplasmic coat on the right side (time at  $37^\circ\text{C}$ : 2 min). *d*, A lysosome containing LDL-ferritin (time at  $37^\circ\text{C}$ : 6 min). Magnifications: *a*,  $\times 58,000$ ; *b*,  $\times 50,500$ ; *c*,  $\times 50,000$ ; *d*,  $\times 40,000$ . Scale bars, 100 nm.

incoming coated vesicles<sup>2</sup>. The rapid sequence of events visualised in the electron micrographs of Fig. 1 precisely parallels biochemically-derived data on the rapid uptake and degradation of  $^{125}\text{I}$ -LDL<sup>10,11</sup>.

### Mutations affecting the LDL receptor

A group of naturally-occurring human mutations has been invaluable in detailing the structure and function of the LDL receptor. Those mutations responsible for the genetic disease called familial hypercholesterolaemia (FH) are most instructive<sup>5,29</sup>. Three distinct mutations seem to occur in the gene for the LDL receptor<sup>6,29,30</sup>. The most frequent mutant allele at the LDL receptor locus is designated  $R^{b0}$ ; it specifies a nonfunctional receptor. Fibroblasts from patients homozygous for this allele ( $R^{b0}/R^{b0}$ ) are unable to bind or take up the lipoprotein, even though they have normal coated pits that form normal coated vesicles<sup>19,30</sup>. A second mutation,  $R^{b-}$ , specifies a receptor that is detectable but binds <10% of the normal amount of LDL. Recently, a rare but extremely informative allele has been identified. This allele, designated  $R^{b+,i0}$ , specifies a receptor that binds LDL but does not allow the lipoprotein to be internalised<sup>11,30</sup>. The failure of internalisation is due to the inability of these abnormal receptors to be incorporated into coated pits. Although coated pits of normal appearance are present, the receptors specified by the  $R^{b+,i0}$  allele are not inside them. Instead the receptors are scattered over noncoated segments of the cell surface<sup>31</sup>.

The hypothesis that the  $R^{b+,i0}$  mutation involves the gene for the LDL receptor implies that the normal receptor must have, in addition to its LDL binding site, a second active site, the internalisation site, which is necessary for the receptor to be recognised as a component of the coated pit. The internalisation site is presumed to be on the cytoplasmic side of the membrane where it can interact with the proteins that constitute the cytoplasmic coat of the coated pit. This formulation implies that

the coat-associated proteins play an active role in the selection of receptors to be incorporated into coated pits and are therefore central to the process of receptor-mediated endocytosis<sup>30,31</sup>.

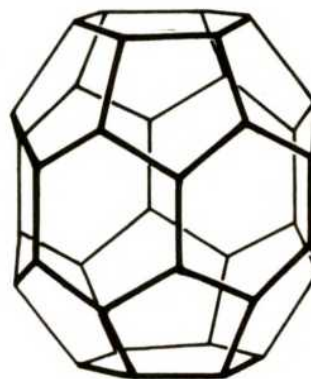
### The structural role of clathrin

Coated pits and coated vesicles were first described in mosquito oocytes by Roth and Porter, who postulated that these structures mediate the uptake of adsorbed proteins from the extracellular fluid during yolk formation<sup>32</sup>. Other investigators, including Fawcett<sup>33</sup> and Friend and Farquhar<sup>34</sup>, found that coated pits and coated vesicles mediate protein uptake in specialised cells of higher animals. Coated pits and vesicles have subsequently been found in virtually all nucleated animal cells.

Coated pits derive their name from their characteristic indented configuration and their fuzzy cytoplasmic coat. Thin section electron microscopy shows that this coat consists of periodically spaced fibres or bristles that radiate from the cytoplasmic surface (Fig. 1a-c). Coated pits have been identified in freeze-fracture electron micrographs where they exhibit a larger number of intramembrane particles than the surrounding plasma membrane<sup>20</sup>. The intramembrane particles in coated pits are approximately 20% larger in diameter than intramembrane particles in non-coated regions of membrane<sup>20</sup>. These findings suggest that coated pits contain a unique set of transmembrane proteins.

In addition to their role in endocytosis, coated vesicles have been implicated in other types of membrane transport within the cell. They are frequently found associated with the Golgi region where they appear to carry enzymes from the Golgi apparatus to the lysosome<sup>34,35</sup>. They also play a part in the secretion of proteins by mammary epithelia<sup>36</sup> and in the retrieval of excess cell surface membrane in presynaptic neurones<sup>37</sup>.

Kanaseki and Kadota first analysed the structure of coated vesicles by electron microscopy using negative staining techniques<sup>38</sup>. They showed that these structures consist of a lipid vesicle surrounded by a basket of protein that is organised in a network of hexagons and pentagons. Pearse developed a rapid method for isolating coated vesicles and made the fundamental observation that the coat of coated vesicles isolated from a variety of animal cells is composed predominantly of a single protein of molecular weight 180,000. She named this protein clathrin<sup>34,39</sup>. Figure 2 shows a stereoscopic view of the proposed structure of the protein network that forms the coat of a coated vesicle.



**Fig. 2** Proposed structure for the network of protein that forms the coat of a coated vesicle. This structure was deduced by Pearse and co-workers from electron microscopic examination of tilted views of negatively stained coated vesicles isolated from porcine brain<sup>34</sup>. The protein coat, which is organised in a network of 12 pentagons and 8 hexagons, is assembled from 108 clathrin molecules that surround a lipid vesicle. Coated vesicles having other sizes and shapes are believed to be constructed similarly with each vesicle containing 12 pentagons plus a variable number of hexagons. Redrawn from ref. 4 with permission.



Clathrin can be removed from coated vesicles by treatment with urea or protonated amines, indicating that it is non-covalently bound to the surface of the membrane<sup>40-43</sup>. With these techniques the protein has now been isolated from brain and chicken oocytes by workers in several laboratories<sup>3,4,28,39-43</sup>, and it has been shown to have important structural properties that are related to its function. For example, in specified *in vitro* conditions, clathrin will spontaneously aggregate to form a basket-like network of hexagons and pentagons similar to those that surround coated vesicles *in vivo*<sup>41-43</sup>.

An antibody to the coat protein of bovine brain coated vesicles (directed primarily against clathrin) reacts *in situ* with coated pits and vesicles in human fibroblasts<sup>28</sup>. The antigen-antibody complex can be visualised by indirect immunofluorescence using light microscopy<sup>28</sup> or by the immunoperoxidase technique in the electron microscope (Figs 3 and 4)<sup>28</sup>. In the electron microscope, the immunoperoxidase-stained clathrin can be shown to underlie coated pits (Fig. 3a), including those that have bound LDL-ferritin (Fig. 4a), as well as coated vesicles (Fig. 3b and c), again including those that have internalised LDL-ferritin (Fig. 4b). Under the light microscope, the coated pits of human fibroblasts show a linear alignment similar to the linear alignment of LDL receptors<sup>28</sup>. However, in other cells, such as Chinese hamster ovary cells, neither the LDL receptors nor the coated pits show a linear distribution, indicating that a linear arrangement is not essential for coated pit function.

### A working model for clustering of LDL receptors in coated pits of human fibroblasts

The genetic, biochemical, and morphological data obtained in the human fibroblast system have been assembled to advance a working model for the mechanism by which LDL receptors cluster in coated pits<sup>1,31</sup>. As described above, the existence of the internalisation mutation implies that the LDL receptor has two active sites—a binding site that must be on the external surface and an internalisation site that is postulated to be on the cytoplasmic surface. By analogy with other transmembrane proteins, the LDL receptor is likely to be synthesised on membrane-bound ribosomes and glycosylated in the Golgi apparatus (Fig. 5). It is then inserted into the plasma membrane at random sites. The receptor migrates laterally in the plane of the membrane until it reaches a coated pit. Interaction of the internalisation site with clathrin (or a clathrin-associated protein) fixes the receptor in the coated pit so that it is carried into the cell when the coated pit invaginates to form a coated vesicle.

Whether receptors reach the coated pits by diffusion in the plane of the membrane or whether they are actively propelled there is not known. Bretscher has suggested that the phospholipids of cell membranes continually flow towards coated pits and coated vesicles<sup>44</sup>. This flow is envisioned to result from the insertion of lipids into the membrane at remote sites with resorption in coated pits and vesicles<sup>44</sup>. If such a flow does exist, it could provide the force that carries selected receptor proteins to coated pits.

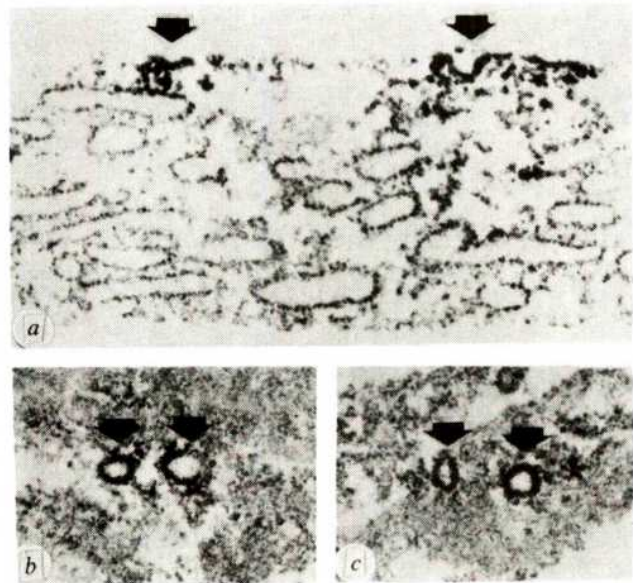
LDL receptors that enter cells in coated vesicles are not destroyed when the vesicles fuse with lysosomes. Rather, kinetic experiments suggest that the receptors return to the surface where they again cluster in coated pits; that is, they are recycled<sup>10,31</sup>. This recycling phenomenon accounts for the fact that fibroblasts ingest saturating levels of LDL at a uniform rate for more than 6 hours even when synthesis of new receptors is blocked by cycloheximide. If recycling did not occur, all of the receptors would be consumed within the first 10 minutes after exposure to LDL.

Morphological and kinetic evidence indicates that insertion of receptors into the membrane, clustering in coated pits, internalisation, and recycling occurs continuously whether or not LDL is present<sup>2,19,31</sup>. In each cycle the LDL receptor spends about 9 min on the cell surface. Assuming that LDL can bind to

the receptor at any time the receptor is on the surface, studies with LDL-ferritin indicate that in the steady state about one-third of receptors are diffusely distributed and two-thirds are clustered in coated pits<sup>2,19</sup>. Thus, in each cycle the receptor spends about 3 min in a diffuse distribution on the surface and about 6 min in coated pits. The length of time between internalisation of the receptor and its reappearance on the surface is not known, but it must be shorter than the time the receptor spends on the surface because no appreciable pool of intracellular receptors is unmasked when binding assays are performed on broken cells<sup>45</sup>. That membrane recycling can be extremely rapid has been shown by studies of the frog neuromuscular junction where the pre-synaptic coated vesicles recycle within seconds<sup>37</sup>.

Other explanations for the morphological and kinetic data in fibroblasts are possible. For example, rather than being recycled it is conceivable that the LDL receptor never actually leaves the surface. That is, as the coated pit pinches off, the LDL is transferred from the receptor to some other carrier with the receptor escaping inclusion in the coated vesicle and thus remaining on the cell surface. While such a mechanism would be consistent with all current data, there is no direct evidence to support it.

The model for insertion of the LDL receptor into coated pits by lateral movement within the plane of the plasma membrane resembles an earlier model advanced for the assembly and secretion of lipid-envelope viruses in animal cells (reviewed in ref. 46). When the vesicular stomatitis virus replicates in cultured animal cells, it produces a messenger RNA for a transmembrane glycoprotein, the G protein, that is synthesised on host membrane-bound ribosomes and inserted at random



**Fig. 3** Electron micrographs showing the localisation of clathrin in human fibroblasts as determined with the indirect immunoperoxidase technique. Monolayers of fibroblasts were fixed with 3% formaldehyde. The membrane was then made permeable with 0.05% Triton X-100, and the cells were incubated first with rabbit anti-coat protein  $\gamma$ -globulin (directed primarily against clathrin) and then with goat anti-rabbit IgG that was coupled to horseradish peroxidase. The cells were then stained for peroxidase as previously described<sup>28</sup>. *a*, Peroxidase-positive indented regions of cell surface membrane (arrows). These regions have the typical shape of coated pits. The normal trilaminar appearance of the membrane has been destroyed by the Triton X-100 preparation procedure. *b*, and *c*, show peroxidase-positive endocytic vesicles (arrows) that have the morphology of coated vesicles. All micrographs are of unstained material. Magnifications: *a*,  $\times 21,500$ ; *b*,  $\times 20,000$ ; *c*,  $\times 21,500$ .



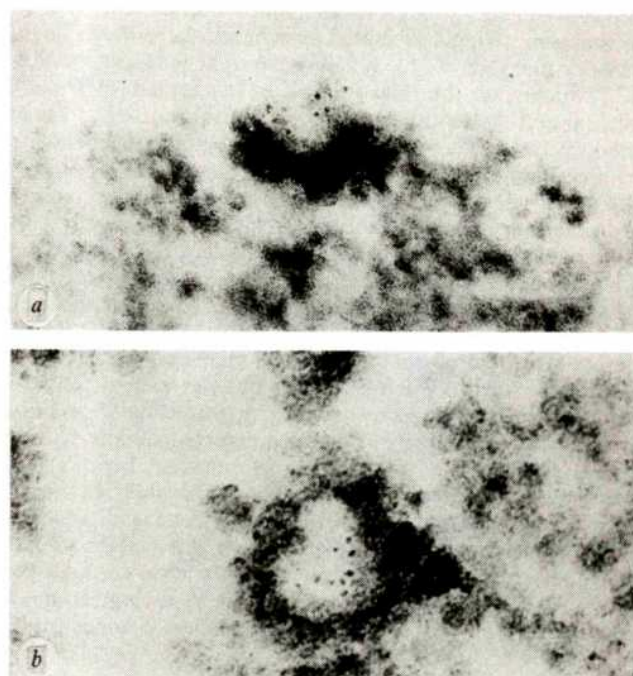
sites into the plasma membrane. The G proteins then gather together to form a patch of plasma membrane that ultimately becomes the envelope of the virus. This clustering of G proteins is mediated by their association with a cytoplasmic protein, the M protein, that is also coded by the viral genome but is synthesised on free ribosomes. The patch of membrane containing G and M proteins serves as the site of attachment of the viral nucleocapsid during viral budding. In the two models, the G protein is analogous to the LDL receptor, the M protein is analogous to clathrin, and the developing patch of viral envelope membrane is analogous to the coated pit. If the two working models prove to be correct, they would suggest a general principle: membrane structures such as viral patches and coated pits are assembled within the plane of the membrane by the interaction of transmembrane proteins with cytoplasmic organiser proteins.

### Variations on a common theme

Table 1 lists the protein ligands for which receptor-mediated endocytosis has now been demonstrated. In each of these systems, uptake of the protein follows rapidly upon binding. In each case the endocytosis is carried out by the same receptor that is believed to mediate the physiologic action of the protein. For six of the systems, including the receptors for LDL<sup>2,19,20,27</sup>, yolk proteins<sup>21,32</sup>, transferrin<sup>22</sup>, epidermal growth factor<sup>23</sup>,  $\alpha$ -2-macroglobulin<sup>24</sup>, and maternal immunoglobulins (IgG)<sup>21,47-49</sup>, internalisation has been shown by electron microscopy to occur in coated pits and coated vesicles. In addition, evidence from the light microscope suggests that insulin enters mouse 3T3 cells through this route<sup>50</sup>. In the other systems listed in Table 1, internalisation via coat pits and coated vesicles has not yet been visually demonstrated. However, in the case of transcobalamin II<sup>14,51</sup>, chorionic gonadotropin<sup>15</sup>, and asialoglycoproteins<sup>16,17</sup>, uptake rapidly follows binding, indicating a close coupling between the two events and in turn suggesting that coated pits may be involved.

In those systems for which coated pits have been documented, the mechanism for receptor clustering into the pits may vary. As described above, LDL receptors seem to be incorporated into coated pits by virtue of their internalisation sites in a process that is independent of LDL binding. One electron microscopic study of epidermal growth factor binding to human fibroblasts suggests that a significant proportion of these receptors behave similarly to those for LDL; even when epidermal growth factor was added in conditions in which lateral diffusion was inhibited by chilling the cells to 4 °C, 34% of the factor bound within coated pits, presumably to receptors that were already located there<sup>23</sup>. On the other hand, light microscopic studies with fluorescent-labelled epidermal growth factor in mouse 3T3 cells suggest that most of the receptors are distributed diffusely when binding is conducted at 4 °C<sup>50</sup>. Receptor clustering in coated pits was observed only after the cells were warmed to 37 °C in the presence of epidermal growth factor. Similar results were reported for fluorescent-labelled  $\alpha$ -2-macroglobulin and insulin in the same cell type<sup>50</sup>. The simplest, but not necessarily the correct, explanation of this discrepancy is that some receptors may reach coated pits as a result of a receptor-mediated mechanism, while others may require a ligand-mediated event.

In three of the systems in Table 1—LDL<sup>10,52</sup>, transcobalamin II<sup>14,51</sup>, and asialoglycoproteins<sup>16,53</sup>—new receptors are efficiently regenerated, probably by recycling of internalised receptors, after the ligand is carried into the cell. As a result, cells internalise these ligands for hours at a steady rate. On the other hand, when epidermal growth factor is incubated with fibroblasts, the initial wave of receptor-mediated endocytosis leads to a rapid 80% depletion in the number of surface receptors<sup>13,53,55</sup>. This reduction has been attributed to a degradation of the internalised epidermal growth factor-receptor complex, which is not accompanied by a reappearance of new receptors. In the continued presence of epidermal growth factor, cells establish a new steady state in which they bind,



**Fig. 4** Electron micrographs showing the binding of LDL-ferritin to clathrin-containing regions of cell membrane. Monolayers of human fibroblasts were chilled to 4 °C and incubated with LDL-ferritin at a concentration corresponding to 22  $\mu\text{g ml}^{-1}$  of LDL-protein for 30 min. The cells were then warmed to 37 °C for 8 min, washed at 4 °C to remove excess LDL-ferritin, fixed with 3% formaldehyde, and made permeable with 0.05% Triton X-100. The cells were then processed for indirect immunoperoxidase localisation of anti-clathrin binding sites as described in the legend to Fig. 3. *a*, Ferritin cores associated with an indented, peroxidase-positive segment of membrane that has the typical morphology of a coated pit, indicating that LDL-ferritin and the antibody directed against clathrin bind to the same region of surface membrane. *b*, Ferritin cores contained within endocytic vesicles that are rimmed by peroxidase reaction product that delineates the clathrin coat. All micrographs are of unstained sections. Magnification,  $\times 100,000$ .

internalise, and degrade the hormone continuously at about 20% of the initial rate. Whether receptor recycling occurs during this steady state is not known.

Whereas the coated pit-coated vesicle seems to be a common portal of entry for receptor-bound proteins, the ultimate destinations of these proteins vary. In seven of the systems in Table 1, the internalised protein (radiolabelled with <sup>125</sup>I in tyrosine residues) is rapidly degraded<sup>6,12-18,25,56-59</sup>. In these systems, no significant amounts of radiolabelled products other than <sup>125</sup>I-monoiodotyrosine are formed by the cells. This suggests the concerted action of multiple proteases and thus implicates lysosomes<sup>56,57</sup>. Moreover, in each of these systems, degradation of internalised protein is blocked by the lysosomal enzyme inhibitor chloroquine<sup>6,13-15,17,25,57</sup>. For LDL<sup>2,52</sup> and epidermal growth factor<sup>23</sup>, lysosomal delivery has been demonstrated directly by electron microscopy.

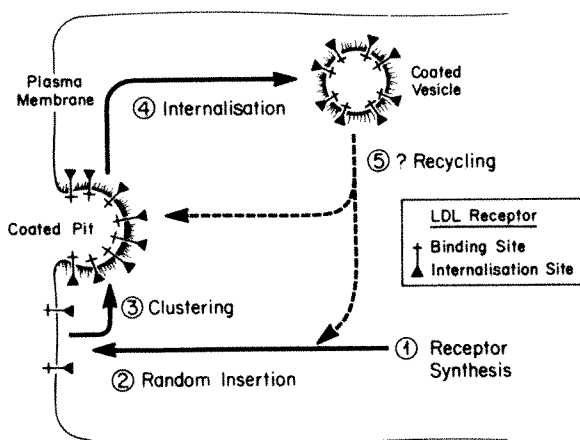
Some of the proteins that enter cells through receptor-mediated endocytosis are not degraded but instead are directed to specific subcellular organelles. For example, yolk proteins accumulate in yolk granules apparently without degradation<sup>21,32</sup>. Nerve growth factor, which enters the cell at the tip of the axon, migrates in vesicles to the cell body where it apparently accumulates in an intact form<sup>62</sup>. Although most of the receptor-bound insulin that enters cells is degraded in lysosomes<sup>12,25</sup>, some fraction of receptor-bound hormone may be delivered to the Golgi apparatus<sup>61</sup> and to nuclei<sup>64</sup>. Lysosomal enzymes that are secreted by cells re-enter the cells through



receptor-mediated endocytosis and accumulate within lysosomes and Golgi-associated structures where they remain active for many days<sup>65-67</sup>. Finally, maternal immunoglobulins (IgG), which enter the fetal yolk sac or the neonatal intestinal epithelial cell at the apical surface, are transported intact in coated vesicles to the basal surface where the IgG molecules are discharged into the fetal or neonatal circulation<sup>21,47-49</sup>.

An important question posed by the above findings relates to the mechanism by which endocytic vesicles and their contents are directed to specific delivery sites within the cells. Do proteins with different destinations enter cells in the same vesicles? If so, how are these proteins sorted and delivered to their separate destinations?

It should be pointed out that receptor-mediated endocytosis is not the only way in which proteins can enter animal cells. At least two other mechanisms exist. First, extracellular proteins are trapped nonselectively in fluid droplets that are internalised by cells through the invagination of noncoated regions of membrane<sup>7</sup>. To date, no receptor-bound protein has been demonstrated to enter cells through such noncoated vesicles. Second, some bacterial and plant toxins and certain viruses appear able to enter cells by direct penetration through the plasma membrane. Direct penetration of physiological molecules, such as protein hormones, has not yet been demonstrated.



**Fig. 5** Working model for the mechanism by which LDL receptors cluster in coated pits on the plasma membrane of human fibroblasts. The postulated steps are as follows: (1) synthesis of LDL receptors on polyribosomes; (2) insertion of LDL receptors at random sites along noncoated segments of plasma membrane, (3) clustering of LDL receptors in clathrin-containing coated pits, (4) internalisation of LDL receptors as coated pits invaginate to form coated endocytic vesicles, and (5) recycling of internalised LDL receptors back to the plasma membrane. It seems likely that the clathrin is also recycled and reutilised. Reprinted from ref. 31 with permission.

## Why do cells need receptor-mediated endocytosis?

The studies cited in this review demonstrate that coated pits and coated vesicles function as transport organelles that carry selected receptor-bound proteins into cells in a tightly controlled fashion. It is easy to understand the need for such a system when cells are dealing with transport proteins such as LDL, yolk proteins, transferrin, and transcobalamin II. The reason for such a sophisticated mechanism of uptake for protein hormones is not yet clear. At the very least, the receptor-mediated uptake and degradation systems serve to degrade the protein hormones so that they, and in some cases their receptors, can act only once. But is this the only reason for receptor-mediated endocytosis of protein hormones? Does the endocytic uptake mediate any of the actions of the hormones? To date, no regulatory action of a

protein hormone (with the possible exception of nerve growth factor) has been shown to require endocytosis of the protein. Moreover, some events, such as insulin-mediated stimulation of glucose transport, occur too rapidly to be accounted for by endocytosis.

To date, only a few studies have addressed the question of whether the long-term biological effects of protein hormones require endocytic uptake. More systems are needed in which the entire sequence of hormone binding and hormone action can be studied by combined biochemical and morphological techniques in isolated cells. Such studies would be facilitated by the availability of cells bearing naturally-occurring or experimentally-induced mutations in the sequence between hormone binding and hormone action. With the process of receptor-mediated endocytosis now in focus and with the powerful tools of cell biology and somatic cell genetics at hand, it seems likely that such systems will be developed and that answers to the questions posed in this review will be forthcoming in the near future.

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# articles

## Non-exponential decay in dielectrics and dynamics of correlated systems

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*A new model for the relaxation of a potential perturbation in dielectric materials is developed based on the correlated properties of a two-level system containing two types of decay mechanism. The time and frequency behaviour of the general relaxation process is shown to be in accord with recent experimental analyses. The technique developed to analyse the model is of general applicability in the solid, and liquid, state.*

It has been shown<sup>1</sup> previously that for materials exhibiting a dielectric loss peak the magnitude of the imaginary part of the susceptibility, as a function of frequency, obeys the empirical relationship

$$\chi''(\omega) = \frac{\omega^m}{(\omega_p^{2s} + \omega^{2s})^{(1-n+m)/2s}} \quad (1)$$

where  $0 \leq m, n$  and  $s \leq 1$ . In the post-peak region,  $\omega > \omega_p$ , this relationship can be written in the form  $\chi''(\omega) \propto (\omega)^{-(1-n)}$  and Jonscher<sup>2-5</sup> has suggested that the behaviour can best be understood as

$$\chi''(\omega)/\chi'(\omega) = \cot(n\pi/2) = \text{constant} \quad (2)$$

which states that the ratio of the energy lost to the energy stored is a constant. In the pre-peak region the imaginary part of the susceptibility exhibits a power law dependence,  $\omega^m$ , and equation (2) is not obeyed. The empirical equation (1) describes a complete decay characteristic for the materials exhibiting loss peaks. It can be Kramers–Kronig transformed to give  $\chi'(\omega)$ , and agreement with experimental measurements has been observed. We use here the presence of a loss peak to define our term dipolar dielectric, and we shall only consider this class of materials.

The universal equation (2) requires the susceptibility to follow a power law in time ( $t^{-n}$ ) in the equivalent, short time, region. It has recently been postulated by Ngai *et al.*<sup>6</sup> that the origin of this form of decay lies in an infrared divergence mechanism and that

the basic requirements for the application of this mechanism in a wide range of systems have been established computationally.

Starting from the concept of an assembly in which the local units possess two equilibrium positions<sup>7,8</sup> (a two-level system) we will show that the consequence of interaction between the local units leads to a complete description of the susceptibility of dipolar dielectrics. The post-peak behaviour arises naturally from this description. The method used is powerful and yields detailed and quantitative information on the microscopic structure and its dynamics. As the final expressions describe the macroscopic behaviour they can be conceived in a variety of ways and reveal the flexibility of the approach presented here. The results of this analysis have a wide range of applicability outside purely dielectric behaviour and similar approaches should be generally applicable.

### The $t^{-n}$ behaviour

Before describing dielectric relaxation in terms of the cooperative model we must describe briefly the origins and requirements of the  $t^{-n}$  behaviour, which is a special case of the time development of transients<sup>9</sup>. Consider a system divided into two interacting sub-systems. The first of these responds rapidly to a stimulus generating a change in the interaction which, in turn, causes a much slower response of the second sub-system. The state of the total system then corresponds to the excited first system together with the unresponded second system, and can be considered as a transient or metastable state which slowly decays as the second system responds. In this way a Franck–Condon progression is developed in molecular spectra<sup>10-12</sup>, the fast system being a high frequency transition which is coupled to the slow response of a discrete spectrum of low frequency oscillations.

The special requirements for a  $t^{-n}$  behaviour are<sup>13</sup>: (1) a continuous spectrum of slow responders extending to zero frequency; (2) a constant density of transition states, per unit energy, for the slow system, in the same energy range; (3) an effective population distribution in the slow states such that they can be regarded as either fully occupied or unoccupied only.



The time behaviour of the state vector of the transient can be obtained from the total hamiltonian  $H$  as

$$\exp(-iHt) = \langle t|0 \rangle \quad (3)$$

which in second order perturbation theory<sup>10,14</sup>, in energy/frequency normalised units, becomes

$$\exp(-iEt) \cdot \exp\{-F(t)\} \quad (4)$$

with  $E$  the unspecified excitation energy of the transient, and

$$F(t) = i n \zeta t - \int_0^t \frac{|VN|^2}{u} \{1 - \exp(-iut)\} du \quad (5)$$

$N$  is the total number of slow responders and  $\zeta$  is their maximum excitation energy, the constant density of states is thus  $N/\zeta$ .  $V$  is the interaction change brought about by the fast excitation and  $u$  the energy difference for nett excitations within the slow response system. Equation (5) is obtained by allowing  $V$  to cause excitations and de-excitations in the slow system, the double sum over initial and final states being replaced by the already completed sum over initial states, for a given energy difference  $u$ , and an integration over  $u$ . This is shown in Fig. 1.

The average energy change per fast excitation will be distributed over the  $N$  slow responders and is  $NV$ . As  $\zeta$  is the maximum excitation energy,  $|NV|^2/(\zeta)^2$  is less than unity and is set equal to  $n$ . Integrating equation (5)<sup>13,15,16</sup> gives

$$F(t) = i n \zeta t - n \{ \gamma + \ln(i \zeta t) + E_1(i \zeta t) \} \quad (6)$$

where  $\gamma$  is Euler's constant and  $E_1(iz)$  an exponential integral<sup>17</sup>. For short times, such that  $t \zeta < 1$

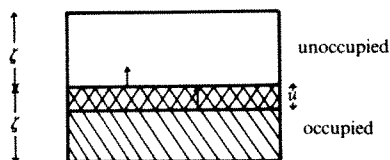
$$F(t) \rightarrow 0 \quad (7)$$

and the transient oscillates with its excitation frequency corresponding to the energy  $E$ . At long times ( $t \zeta > 1$ )

$$F(t) = \exp\{-i(E - n \zeta)t\} \cdot \exp(-n \gamma) \cdot \exp(i n \pi / 2) \cdot (t \zeta)^{-n} \quad (8)$$

where the excitation energy of the transient has been reduced by  $n \zeta$ , and the transient decays as  $t^{-n}$ .

In applying this result to our problem the fast excitations and the slow responders are assumed to lie in the same continuum of states, the slow responders merely being slower than a particular fast transient excitation within the same total system. The system returns to its unexcited state by means of excitations of the slow responders, which decay monotonically. For this reason  $E$  is taken equal to  $n \zeta$ , and it has already been shown that  $n$  lies between zero and unity corresponding to no excitation or total excitation of the system respectively. Note that any observable property of the transient follows a behaviour governed by the real part of equation (4), and thus oscillates with diminishing frequency while decaying as  $t^{-n}$ .



**Fig. 1** The two-level system: the range of initial states available for excitation with energy  $u$  is shown by cross hatching. The equivalent number of initial states is  $(Nu/\zeta)$  where  $N/\zeta$  is the constant energy density.

## Interacting two-level systems

A local two-level system can be considered as a group of atoms, ions or molecules possessing two equilibrium configurations separated by a small energy difference and between which local tunnelling is allowed<sup>7,8</sup>. Such a system can be described by a spin

formalism in which the energy tensor is

$$\begin{pmatrix} B_e & U_e \\ U_e & -B_e \end{pmatrix} \quad (9)$$

where  $B_e$  is half the energy difference between local potential minima and  $U_e$  is the off-diagonal tunnelling (transition) element. Such systems can couple to elastic deformations and have been used to describe a range of glass properties<sup>18</sup>. When the local system possesses a dipole moment the coupling to an electric field takes the form

$$\begin{pmatrix} d & \mu \\ \mu & -d \end{pmatrix} F \quad (10)$$

with the off-diagonal elements  $\mu$  allowing a resonance absorption, the diagonal elements a relaxation spectra. A spin-spin interaction arises from the coupling of pairs of local spin systems through the off-diagonal elements of the tensor in equation (9). These interact with phonons and give the usual dipolar spin-spin interaction through a virtual two-phonon exchange mechanism<sup>19</sup>. The spin-spin interaction can be regarded as a perturbation on an unperturbed local system with energy levels  $\pm B_e$  together with a local transition interaction. It contains three types of term, each of which has a definite and unique influence on the overall system.

(1) The secular interaction: this is a dipole-dipole cooperative interaction which contributes to the energy of each unperturbed spin. It is the basis of the Ising hamiltonian and when calculated in the mean field approximation has a contribution to  $B$  of  $T_c M$ , where  $M$  is the mean value of the  $z$  component of the local dipole unit vector, in this case electric, and  $T_c$  is a characteristic parameter of the system. The system can no longer be regarded as a set of local systems because of the cooperative interaction. Its energy levels are macroscopic and the macroscopic thermal average,  $M_e$ , of  $M$  is

$$M_e = \tanh\left(\frac{B + k T_c M_e}{k T}\right) \quad (11)$$

which is a consequence of the condition that the dipoles can only be in one of two states.  $B$  is the average of  $B_e$  over all the system, and the dipole of the system in equilibrium is  $N M_e d$ . The value of  $M_e$  is defined by equation (11) and is determined from  $B$ ,  $T_c$  and the temperature  $T$ . The energy  $B$  can be regarded as the splitting of a two-level system by a well-defined internal field. In amorphous glassy systems the local value of  $B_e$  is not constant but takes a continuous range of values with a constant number density at each value<sup>19,20</sup>. It has been suggested<sup>6</sup> that this applies to a wide variety of materials.

(2) The flip-flop interaction: this interaction allows a pair of dipoles to exchange spins synchronously and without altering  $M$ . Local spins move through the whole system by this mechanism. The time taken to complete a spin exchange is

$$t_e \approx \pi / V_{ff} \quad (12)$$

where  $V_{ff}$  is the flip-flop interaction energy. The time has been estimated as  $10^{-8}$ – $10^{-10}$  s (ref. 19).

On a time scale greater than  $t_e$  the local spins will sample the whole of the local values of  $B_e$ . In general those local spins whose values of  $B_e$  differ by less than the maximum value of the interaction energy,  $V_{ff \max}$ , can be regarded as in resonance and hence involved in a true spin-spin exchange. Those spins with a greater difference than  $V_{ff \max}$  interact in an off resonance manner and do not have a true spin-spin exchange.

In this way, on a time scale greater than  $t_e$ , the macroscopic value of  $B$  in equation (11) will alter without altering  $M_e$ . But the nature of equation (11) requires an alteration in  $B$  to generate a consequent change in  $M_e$ . Thus the flip-flop interaction has to be regarded as causing fluctuations in  $M_e$  about a well defined average.

(3) The raising and lowering interaction: this interaction couples  $M$  with raising and lowering operators. These operators allow excitation and de-excitation of the unperturbed local

system, and generate absorptions at multiples of the Larmor frequency in nuclear magnetic resonance spectra<sup>21</sup>. When paired together the value of  $M$  is unchanged and oscillations are generated at frequencies equal to the difference between excited and unexcited local systems<sup>22</sup>. Since the frequencies span the range from zero up to a particular maximum they can be regarded as a system of slow responders.

All spins will be connected by this interaction, hence a fluctuation such as a flip-flop interaction will create a transient which decays as  $t^{-m}$  as the frequencies less than  $V_{ff \max}$ , that is,  $10^8$ – $10^{10}$  Hz, respond. The magnitude of the parameter being given by

$$m = (V_{ff \text{ average}} / V_{ff \max})^2 \quad (13)$$

## The rate equation

The fundamental concepts required to establish the framework of our approach have already been established. The basic model is that a deviation from equilibrium in a macroscopic system resulting from the application or removal of an external field is restored to an equilibrium that is itself fluctuating on a long time scale. Because of the connection between  $M$  and  $B$  in equation (11) fluctuations in the ground state energy  $B$  must affect the rate of restoration of equilibrium. Calculations using this model are applicable to many fields but we shall consider here a dipolar dielectric.

The dipolar dielectric is represented by a double minimum in the total (macroscopic) free energy as indicated in Fig. 2. Each minimum refers to a set of configurations of local dipoles with their orientations effectively in one of two alternative directions. Each configuration is thus specified by a set of local dipole orientations, simultaneously fully occupied, which have an equal number of unoccupied levels belonging to a configuration with an opposing dipole orientation and separated from them by a large potential barrier. The two sets of configurations are therefore only accessible to each other either by thermally activated or tunnelling processes. Thermal equilibrium is established between groups of macroscopic configurations rather than independent local orientations.

After the removal of an externally applied perturbing influence there are two competing relaxation processes as well as the fluctuations to consider. These are thermal activation and local tunnelling, (Fig. 2c and b).

The rate equation for the thermal process can be written as<sup>23</sup>

$$\frac{dM}{dt} = -\nu_E \cosh\left(\frac{B + kT_c M}{kT}\right) \left\{ M - \tanh\left(\frac{B + kT_c M}{kT}\right) \right\} \quad (14)$$

where  $\nu_E$  has an activated form with a pre-exponential frequency of the order of  $10^{13}$  Hz, that is

$$\nu_E = \nu_0 \exp(-\Delta/kT) \quad (15)$$

Writing

$$M = M' + \tanh\left(\frac{B + kT_c M_c}{kT}\right) = M' + M_c \quad (16)$$

a linear form of equation (14), exact in  $M_c$ , can be found,

$$\begin{aligned} \frac{dM'}{dt} &= -\nu_E \cosh\left(\frac{B + kT_c M_c}{kT}\right) [M' \{1 - (1 - M_c^2) T_c / T\}] \\ &= -\omega_p M' \end{aligned} \quad (17)$$

where  $M'$  is the deviation from equilibrium of  $M$ .

As  $\omega_p$  involves  $M_c$  it can be considered as an expectation value of an operator in the fluctuating system. Thus the rate itself fluctuates and must be averaged over these fluctuations in  $\omega_p$ . To carry out this averaging, the form of the equilibrium fluctuations must be determined.

Resonance flip-flops exchange dipoles between the two minima, in each of which a new configuration is generated. The initial equilibrium value of  $B$  of the macroscopic state is not appropriate to the new configurations and the state evolves in

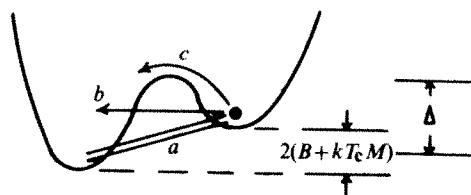


Fig. 2 A double minima potential. a, A tunnelling process of synchronous excitation and de-excitation. b, A cooperative tunnelling relaxation process. c, A thermally activated relaxation process.  $\Delta$ , the average energy of the maximum above the two minima.

time to accommodate the change. The state of the configuration in each minimum immediately following a flip-flop will effectively be that due to the excitation of a dipole from a fully occupied to a non-occupied level. The population criterion (3) for the slow responders is completely fulfilled and the time evolution of each configuration, and hence the system, follows a time power law, the exponent of which has been defined as  $m$ .

During the transient decay the requirement of a well defined average necessitates a balance which arises from the multiply-connected pairs between the two configurations of the transient, generating a new equilibrium state of the system that satisfies equation (11).

The fluctuations must therefore follow a time development of the form

$$(t - t_1)^m \cdot t_1^{-m} \quad (18)$$

as the balancing is delayed in time by  $t_1$  to allow for the initial decay. Equation (18) represents fluctuations about a well defined average expectation value because of the constant time average

$$t^{-1} \int_0^t (t - t_1)^m \cdot t_1^{-m} dt_1 = \Gamma(1 + m) \cdot \Gamma(1 - m) \quad (19)$$

To allow for these fluctuations a time average has to be taken in the rate equation, equation (17),

$$\left\langle \frac{dM'}{dt} \right\rangle \propto -\omega_p(t)^{-1} \int_0^t (t - t_1)^m t_1^{-m} M'(t - t_1) dt_1 \quad (20)$$

The evolution of the initial state,  $t_1^{-m}$ , competes with the thermal decay process, but the balancing  $\{(t - t_1)^m\}$  initiated by the decay at  $t_1$  generates a new macroscopic state satisfying the initial conditions and thus initiates a new contribution to the relaxation, giving the composite relaxation rate at time  $t$  shown in equation (20). The value of  $M'(t - t_1)$  is evaluated from

$$\frac{d\{M'(t_s - t_1)\}}{d(t_s - t_1)} = -\omega_p(t_s - t_1)^m t_1^{-m} M'(t_s - t_1) \quad (21)$$

giving

$$\ln[M'(t_s)/M'_{(0)}] = -\omega_p \int_0^{t_s} (t_s - t_1)^m t_1^{-m} d(t_s - t_1) \quad (22)$$

and hence, using equation (20), and normalising to unity,

$$M'(t - t_1) = M'_{(0)} \exp\{-\omega_p(t - t_1)\} \quad (23)$$

The variable in equation (21) is the time span,  $(t_s - t_1)$ , during which the relaxation proceeds uninterrupted as opposed to the actual time,  $t$ , at which measurements are made.

The initial deviation from equilibrium,  $M'_{(0)}$ , arises from the removal of a perturbation which alters  $B$ , for example an external electric field which makes a contribution  $Fd$  to  $B$  where  $d$  is the local  $z$  component of the dipole moment, and is given by

$$M'_{(0)} = \tanh\left(\frac{B + Fd + kT_c M'_{(0)}}{kT}\right) - M_c \quad (24)$$

That is,  $F$  rotates the macroscopic dipole towards or away from the fixed internal field direction.

The rate constant  $\omega_p$  is the maximum probability that a thermally activated process leads to a transition of a dipole between alternate minima. The activated factor is the probability for a thermal process of sufficient energy to surmount the barrier, and the pre-exponential factor is the quantum mechanical transition rate at the barrier peak.

A second independent relaxation process, that of local tunnelling, may also occur. Each tunnelling event takes place in a time of approximately  $\nu_0^{-1}$  and flips a dipole from one minimum to the other. A transient configuration is thus created in each well in a manner similar to that generated by flip-flops, and which decays by a time power law with a different exponent,

$$\cos(n\pi/2) \cdot (t\zeta)^{-n} \quad (25)$$

the range of frequencies,  $\zeta$ , in the slow system being from zero up to  $\nu_0$ , or the maximum value in the system, whichever is lower. This behaviour describes the tunnelling relaxation of a deviation in  $M$ , and in this case there is no balancing time behaviour. The magnitude of  $n$  is proportional to the amount of configuration change introduced by exciting a single dipole, as a fraction of the maximum possible change, and is therefore the degree of cooperation of dipole tunnelling.

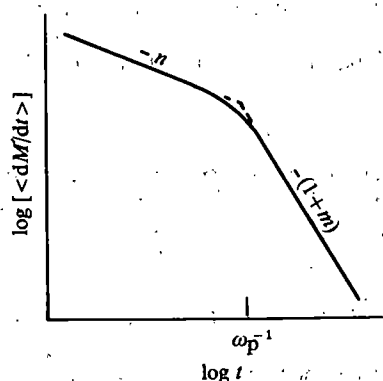


Fig. 3 A log-log plot of the decay current as a function of time. The power law limiting behaviour regions can be clearly seen.

This cooperative tunnelling relaxation process competes independently with the thermally activated relaxation, equation (23), and takes place on the same time scale. Therefore, at a time  $t-t_1$  after the relaxation has been initiated at  $t_1$ ,  $M'$  has relaxed to

$$M'(t-t_1) = M'_{(0)} \cos(n\pi/2) \cdot [\zeta(t-t_1)]^{-n} \exp\{-\omega_p(t-t_1)\} \quad (26)$$

The value of the relaxation current observed at time  $t$  is that given in equation (20) with  $M'(t-t_1)$  given in equation (26) and is

$$\begin{aligned} \left\langle \frac{dM'}{dt} \right\rangle &= \frac{\omega_p \cos\left(\frac{n\pi}{2}\right) \zeta^{-n} \int_0^t (t-t_1)^{n-1} M'_{(0)} (t-t_1)^{-n} e^{-\omega_p(t-t_1)} dt_1}{\int_0^t (t-t_1)^{n-1} dt_1} \\ &\propto \omega_p \cos\left(\frac{n\pi}{2}\right) \zeta^{-n} M'_{(0)} e^{-\omega_p t} t^{-n} {}_1F_1(1-m; 2-n; \omega_p t) \end{aligned} \quad (27)$$

where  ${}_1F_1(;;)$  is the confluent hypergeometric function<sup>24</sup>. Equation (27) describes a complex situation. An initial deviation decays through two independent processes. One is an internal readjustment, and is described by the  $t^{-n}$  behaviour. The other is a thermal decay process in which the rate constant fluctuates about an average value. These fluctuations are the result of a

second microscopic interaction. The composite rate observed at time  $t$ , therefore, has to be averaged over the fluctuations. A linear Ising model calculation of configuration correlation functions<sup>25</sup> has revealed some of the features of the above expression.

## Frequency-dependent susceptibility

To obtain the linear frequency-dependent susceptibility a form of  $\langle dM'/dt \rangle$ , equation (26), requires to be determined in which the initial deviation from equilibrium is linear in field strength. Expanding equation (24) gives

$$M'_{(0)} = \frac{Fd}{kT} (1-M_e^2) \{1 - (1-M_e^2)T_c/T\}^{-1} \quad (28)$$

The frequency dependence of the susceptibility is given by a standard transformation of equation (26). The time development of equation (26) is shown in Fig. 3. At short times the confluent hypergeometric function has a limiting value of unity, as has the exponential term, and hence

$$\left\langle \frac{dM'}{dt} \right\rangle = J \propto (\omega_p t)^{-n} \quad (29)$$

where  $J$  is the decay current. At infinite time the asymptotic form of the hypergeometric term is proportional to  $\exp(+\omega_p t) \cdot (\omega_p t)^{n-m-1}$  and the equivalent current is given by

$$(\omega_p t)^{-(1+m)} \quad (30)$$

In the limited region around  $t \approx \omega_p^{-1}$  the decay is dominated by the exponential term. The Laplace transform of equation (26) is<sup>26</sup>, in normalised form,

$$\chi(\omega) \propto \frac{\omega_p^{1-n}}{(\omega_p + i\omega)^{1-n}} \cdot {}_2F_1\left(1-n, 1-m; 2-n; \frac{\omega_p}{(\omega_p + i\omega)}\right) \quad (31)$$

in which  ${}_2F_1(;;)$  is the gaussian hypergeometric function<sup>26</sup>. Equation (31) has the simple asymptotic behaviour that at frequencies greater than  $\omega_p$  both the real and imaginary parts of the complex susceptibility are proportional to  $\omega^{-(1-n)}$ , in agreement with equations (1) and (2). At frequencies less than  $\omega_p$  the imaginary part of the susceptibility is proportional to  $\omega^m$  and the real part is given by  $\chi'_{(\omega=0)} - A\chi''_{(\omega)}$  where  $A$  is a constant, in agreement with equation (1). The curvature parameter  $s$  of equation (1) is found to be a single-valued function of  $m$  and  $n$ .

The particular value of  $m$  equal to unity is of interest as it can arise either when there is perfect correlation in the flip-flop processes, or when measurements are made at sufficiently high frequencies that the slow responders cannot give rise to the fluctuation process. Both forms have been observed experimentally<sup>1</sup>.

## Discussion

The basic requirements for the present model is the presence of a set of two-level systems in which the frequency difference ranges from zero upwards, and in which the number density is effectively constant. The existence of these states has been demonstrated experimentally in glasses<sup>19</sup> and theoretically established in other materials<sup>6</sup>; from this the high frequency behaviour follows. It should be pointed out that most dielectric susceptibility measurements are made close to a phase transition<sup>27,28</sup> where a cooperative system of structural changes with at least two local potentials must exist. The local two-level system considered here can be regarded as describing the structural changes involved in a phase transition. The processes described here are the microscopic reality behind Jonscher's 'screened hopping model'<sup>29</sup>. The exponents  $n$  and  $m$  have been determined as follows;  $m$  is the degree of structural adjustment required for the average flip-flop process. It is therefore a correlation of these processes in the ground state.  $n$  is the degree



of structural adjustment required for the average spin flip (spin raised or lowered). It is thus a correlation factor for the dynamic restorative tunnelling events.

It is expected that mechanical strain, nuclear magnetic resonance  $T_1$  measurements<sup>30</sup>, magnetic susceptibility and the molecular dynamics of plastic crystals will show similar behaviour<sup>28</sup>. In particular ultrasonic absorption measurements in which the pseudo-spins respond to variations in  $B$  produced by acoustic vibrations show a similar response<sup>31</sup>.

Finally we point out that a non-exponential decay behaviour is a theoretical necessity in all systems which do not have an infinite range of energy in their decomposed state<sup>32</sup>, for example which may recombine. The required behaviour has to be faster

than exponential at short times and slower than exponential at long times. The transitional behaviour between these regions is close to exponential in form, as it is here. Usually the available experimental time region is such that only minor deviations from an exponential behaviour can be observed. Because dielectric susceptibility is observable over wide ranges of frequency and amplitude, and is amenable to temperature scaling, it provides a unique opportunity to study the details of the non-exponential decay and the microscopic mechanisms involved.

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# Thermal aspects of komatiite generation and greenstone belt models

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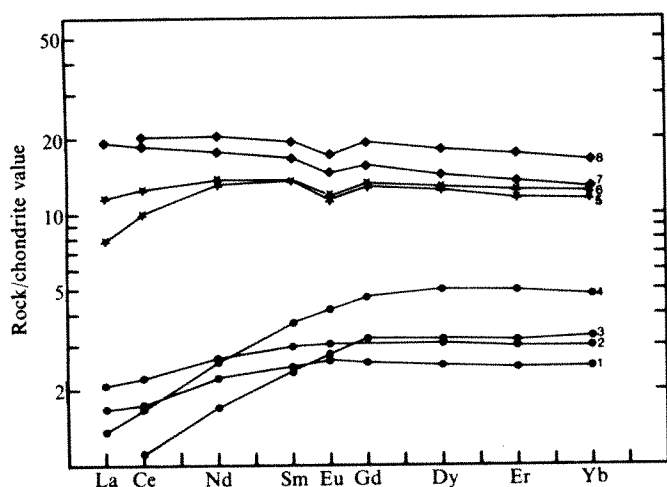
*Thermal modelling suggests that the problems posed by the high liquid temperatures ( $\sim 1,650^\circ\text{C}$ ) of peridotitic lavas in Archaean greenstone belts, and the implied high degree of mantle melting ( $\sim 70\%$ ), are significantly reduced by considering uprise of a more refractory mantle diapir having an inherent density contrast with the surrounding mantle, and in a tectonic environment analogous to a marginal basin.*

LAVAS of komatiitic composition<sup>1,2</sup>, representing very high magnesian liquids with up to 33% MgO, are a common and distinctive magma type in Archaean greenstone belts, although comparable high magnesian liquids become increasingly rare in younger post-Archaean volcanic provinces. They occur most frequently in the lower parts of greenstone volcano-sedimentary sequences, where there may be several cycles of ultramafic-mafic lavas. Although crystal fractionation has been demonstrated in some ultramafic-mafic lavas<sup>3</sup>, the presence of spinifex quench textures<sup>4</sup> indicates that in most cases the high-MgO character is primary and not due to olivine accumulation. Deriving such highly magnesian liquids by mantle fusion poses severe thermal problems.

Experimental studies<sup>5</sup> have shown that the high liquidus temperatures of peridotitic komatiite ( $\sim 1,650^\circ$  at 1 atm) would require  $\sim 70\%$  partial melting of mantle pyrolite. Similar melting experiments on mantle nodules<sup>6</sup> have also demonstrated

that high degrees of melting would be required to produce peridotitic komatiite liquids. Modelling of the thermal evolution of ascending pyrolite diapirs<sup>7,8</sup> indicates that attainment of this high degree of partial melting would necessitate initiation of mantle diapirs from depths in excess of 300 km. This somewhat extreme requirement has led to suggestions<sup>8</sup> that the mantle source regions may have been enriched in radioactive heat-producing elements.

Recent detailed geochemical studies of Archaean greenstone volcanic sequences in Canada, Australia, Rhodesia, South Africa and Finland<sup>9-12</sup> have shown, however, that many peridotitic komatiite lavas not only have low trace element abundances, as may be expected with high degrees of mantle melting, but also have light rare-earth depleted rare-earth element patterns and low incompatible element abundances (Fig. 1). These features have been taken as indicating that such peridotitic komatiites are derived from a 'depleted' mantle source<sup>12</sup> similar to that for modern mid-ocean ridge basalts, and are most unlikely to have been enriched in U, Th, K and Rb. Other peridotitic komatiites may have essentially undepleted or even slightly enriched geochemical characteristics<sup>11</sup>, but these are not a dominant group. The geochemical studies have also demonstrated that associated tholeiitic basalts in greenstone sequences have flat chondritic rare-earth patterns (Fig. 1), higher levels of incompatible elements and essentially 'undepleted' incompatible element ratios. They do not seem to be consanguineous with the depleted komatiitic lavas but have been derived from a different mantle source. Inhomogeneity in the Archaean mantle



**Fig. 1** Selected rare-earth patterns of depleted peridotitic komatiite (●); basaltic komatiite (★); and tholeiitic lavas (◆) from Australian, Rhodesian and North American greenstone belts. Data sources: Patterns 1, 2, 4, 5, 6, and 7 ref. 11; pattern 3, ref. 9; pattern 8, ref. 36. See also ref. 12.

is indicated<sup>11,12</sup>. The thermal problem of accounting for the high degree of melting of the more depleted mantle source is, however, exacerbated.

### Thermal aspects

Cawthorn's<sup>8</sup> modelling of the thermal budget of ascending mantle diapirs was based on estimated thermal parameters for pyrolite (undepleted mantle). We have repeated these calculations using a depleted, more refractory peridotite (which has had a basalt fraction extracted), and the results are shown in Table 1. A depleted, more magnesian peridotite not only has a higher 1-atm solidus temperature, but also has higher values than pyrolite for the latent heat of fusion and for the heat capacity. Estimates of these quantities have been made, and although the absolute values for the chosen parameters are uncertain, they do illustrate the relative difference in melting behaviour of a pyrolite and a more refractory mantle diapir. As emphasised by Cawthorn<sup>8</sup>, the latent heat of fusion exerts a large buffering effect on the degree of mantle melting. In fact, on the basis of recent thermochemical data<sup>13</sup>, the values used by Cawthorn for the latent heat of fusion may be rather low, accentuating the thermal problem of deriving komatiitic liquids from pyrolite diapirs. Similarly the value assumed for the latent heat of fusion of depleted mantle may be an underestimate, but was chosen only to be of the correct magnitude for direct comparison with Cawthorn's modelling.

For both models the mantle source region is assumed to be at the same initial temperature. Note, however, that the more magnesian depleted peridotite would be of lower density and significantly more buoyant than the pyrolite<sup>14</sup>, and may even begin to rise at temperatures below that of surrounding and overlying undepleted mantle<sup>14</sup>. Because the depleted peridotite solidus is 200 °C higher than that of pyrolite, a depleted peridotite diapir must undergo more adiabatic uprise in the solid state before intersecting the solidus than would a pyrolite diapir derived from the same depth. This has been accommodated in the modelling. Nevertheless, Table 1 shows that a refractory mantle model allows a higher 1-atm liquid temperature with a smaller degree of partial melting than does the pyrolite model; moreover, the Mg contents of the erupted liquids (calculated on the basis of the method of Hanson and Langmuir<sup>15</sup>) are also higher.

The depleted peridotite model therefore makes it possible to obtain the required liquidus temperature (~1,650 °C) by initiation of diapirism from shallower depths and with a smaller degree of partial melting than allowed by the pyrolite model. At the same time the depleted peridotite model has a built-in buoyancy factor able to initiate diapirism. Whether these features can be reconciled with a viable tectonic model for greenstone belts remains to be determined.

### Tectonic models

In seeking an acceptable tectonic model for greenstone belt formation it is necessary to account for the field, tectonic and chronological relationships of the various rock types, the geochemical characteristics of the lava sequences and associated plutons, and the thermal problems of komatiite generation. A wide variety of uniformitarian and non-uniformitarian models have been proposed<sup>1,16-22</sup>, although few consider the thermal constraints on the generation of komatiites. On the basis of heat flow modelling, Bickle<sup>23</sup> has suggested that subduction did occur in the Archaean, with sub-lithospheric heat flow of two or three times the present values and involving rapid production of lithospheric plates of comparable thickness to those at present. If subduction did take place, then the depleted mantle cap to the subducting lithosphere is an obvious source of depleted refractory mantle with the potential for diapiric uprise. Indeed Oxburgh and Parmentier<sup>24</sup> have suggested this as a probable mechanism for back-arc spreading and the formation of marginal basins. Here we extend this concept to link with earlier proposed marginal basin models for greenstone belts<sup>22</sup>.

The essence of the Oxburgh and Parmentier<sup>14,24</sup> model is that the depleted lithosphere with its basaltic capping sinks into the mantle until it heats up to or near the temperature of the surrounding mantle. At this point it segregates from the dense eclogite cap and rises diapirically because there is a significant

**Table 1** Ascending peridotite diapirs: % partial melting and temperatures and MgO contents of liquids

Initial <i>P</i> , <i>T</i> and depths of diapir			Pyrolite model <sup>8</sup>			Depleted peridotite model		
<i>P</i> (kbar)	Depth (km)	<i>T</i> (°C)	Final <i>T</i> (°C)	% Melt	% MgO*	Final <i>T</i> (°C)	% Melt	% MgO*
50	150	1,700	1,372	29	21	1,513	19	31
75	225	1,950	1,497	47	32	1,651	42	44
100	300	2,200	1,606	69	42	1,780	63	53

Parameters used in the modelling are:

Heat capacity of solid (cal g <sup>-1</sup> °C <sup>-1</sup> )	Pyrolite model <sup>8</sup>	Depleted peridotite model
Heat capacity of liquid (cal g <sup>-1</sup> °C <sup>-1</sup> )	0.30	0.35
Latent heat of fusion (cal g <sup>-1</sup> )	0.50	0.55
	100	120

Both models assume an adiabatic cooling rate of 1 °C kbar<sup>-1</sup>, with gravitational heat loss retained in the diapir of 0.4 cal g<sup>-1</sup> kbar<sup>-1</sup>. In the pyrolite model<sup>8</sup> the pyrolite solidus is taken to be at 1,200 °C at 1 atm, with d*T*/d*P* of 10 °C kbar<sup>-1</sup> with a nonlinear melting interval. The depleted peridotite model assumes a solidus at 1 atm, of 1,400 °C, again with d*T*/d*P* of 10 °C kbar<sup>-1</sup> but with a constant linear melting interval of 600 °C. Higher heat capacity and latent heat of fusion values based on mineral data in ref. 13.

\* Modelling of MgO abundances in liquids similar to that of Hanson and Langmuir<sup>15</sup>, using 1-atm distribution coefficients. Note quoted MgO values are in cation mol %. Pyrolite<sup>7</sup> has 48 cation mol % MgO; depleted peridotite assumed to have 53 cation mol % MgO (for reference, sample 49J from the Barberton belt<sup>1,5</sup> has 32% MgO equal to 42 cation mol %).

density difference ( $0.06\text{--}0.09\text{ g cm}^{-3}$ ) from the surrounding more Fe-rich undepleted mantle. The rising diapir will at some time intersect the refractory mantle solidus and thereafter undergo progressive fusion. The rising diapir may cause fusion of the normal mantle surrounding it (this is especially true where intrinsic buoyancy is a significant driving force in diapiric uprise<sup>25</sup>), allowing potential mixing of mantle sources or magmas. Thus the geochemistry of the erupted magmas could conceivably vary from 'depleted' to 'enriched' and need not directly reflect the composition of the diapir itself. The first magmas erupted would be peridotitic komatiite, but perturbation of the mantle by the diapir and dissipation of its remaining thermal energy would cause extensive basaltic volcanism characteristic of many greenstone sequences. Because this type of diapirism is closely linked to subduction and hence to zones of crustal generation, the general association of rock types would be similar to that proposed for the *rocas verdes* marginal basin model of Tarney *et al.*<sup>22</sup>.

The present model, however, allows considerable improvements to be made to the *rocas verdes* model, particularly towards explaining the lack of rifting of the lithosphere (and consequent spreading), the occurrence of multiple greenstone belts, repetitions of the volcanic cycle and the lack of modern equivalents of komatiitic lavas.

An important feature of the model is that the driving force is the intrinsic density contrast rather than a thermally dependent density contrast. The rate of diapiric uprise depends on both the size of the diapir and the viscosity of the surrounding mantle. High heat flow in the early Archaean<sup>23</sup> would mean that the mantle had a low viscosity, thereby enabling smaller diapirs to rise, with perhaps more than one pulse, leading to repeated volcanic sequences. However, the viscosity of the mantle will increase with time as the geothermal gradient falls as a consequence of crustal growth and the transfer of radioactive elements U, Th, K and Rb into the crust. Conversely the upper mantle becomes progressively more depleted and refractory (as evidenced by Nd and Sr isotope data<sup>26</sup>), lessening the density contrast with the subducting lithosphere. Thus only increasingly larger diapirs would be able to rise. The implication is that earlier greenstone belts might be smaller (for example, Barberton and Pilbara greenstones) with perhaps more repeated volcanic cycles, whereas later belts would be larger and more linear (for example, Superior Province, Yilgarn Block). Small diapirs might thin the crust and promote basin formation, but would not necessarily rupture the lithosphere. Eventually the density contrast would become too small and the viscosity too high to allow refractory mantle diapirs to migrate to higher levels. A corollary of this is that there may be no (or very rare) modern equivalents of komatiitic lavas. Alternatively, only very large diapirs would be able to rise; these, however, would rupture the lithosphere and generate seafloor spreading similar to that in modern back-arc marginal basins. It is interesting that high-magnesian (up to 18% MgO) lavas and dykes are known from several marginal basin ophiolite complexes, including the *rocas verdes* complex in southern Chile<sup>27</sup>.

The existence of multiple greenstone belts in younger Archaean provinces (for example, Superior Province, Yilgarn Block) poses problems for the conventional marginal basin model, bearing in mind the time constraints from these areas. Recent isotopic studies<sup>28</sup> have indicated that most of the Superior Province, including the greenstone belts, formed within a restricted time span of 100–200 Myr. This implies extremely rapid crustal generation and penecontemporaneous formation of the greenstone belts. Similar evidence exists from the Yilgarn Block<sup>29</sup>. One possibility is that the subduction zone migrates oceanwards as the newly generated crust accretes laterally and that a series of greenstone belts are formed in sequence, each generated by individual diapiric events. A modern parallel is the Mariana Arc system where a series of remnant arcs and intervening marginal basins have been formed since the mid-Tertiary as the active arc itself has moved eastwards<sup>30</sup>.

However, it is also possible that there is a small but significant hiatus between the generation of crust and formation of greenstone belts, and that the latter were all formed simultaneously. Cox<sup>31</sup>, for instance, has used the Oxburgh–Parmentier model<sup>24</sup> to account for the extensive early Mesozoic Karroo volcanism in southern Africa. He suggests that continued subduction of Pacific lithosphere along the western margin of Gondwanaland during the late Palaeozoic led to a substantial build-up of low-density refractory mantle in the 'back-arc' region beneath the continent, and that it was the eventual diapiric rise of this material into the more fertile mantle above that provided the thermal energy for the extensive Karroo-related volcanism. For this to be a feasible mechanism requires a fairly shallow subduction zone in order to carry depleted lithosphere well beneath the continent. Shallow subduction beneath the Andes is associated with voluminous calc-alkaline volcanism and plutonism, while equivalent subduction beneath the active basaltic island

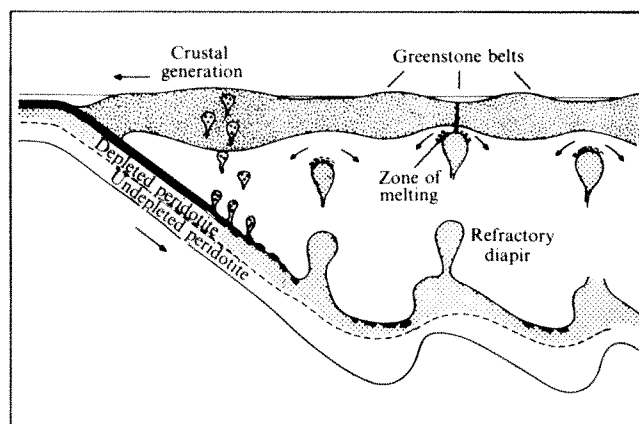


Fig. 2 Schematic representation of back-arc diapirism in a region of rapid lateral crustal growth. Possible model for multiple greenstone belts is shown.

arcs and marginal basins of the Western Pacific is located along steeply inclined Benioff zones. Molnar and Atwater<sup>32</sup> have ascribed this difference to the age of the subducting lithosphere: older, cooler lithosphere in the Western Pacific sinking back steeply into the mantle while younger warmer lithosphere subsides more gently. The high heat flow in the Archaean coupled with rapid plate production and shorter convection cells would favour shallow subduction and, by analogy with the Andes, extensive crustal generation. The refractory subducted lithosphere could rise diapirically at several points under a continent to produce multiple (and ensialic) greenstone belts (Fig. 2). Provided they originated at depths in excess of 200 km, they could generate high-temperature lavas with the chemistry of peridotitic komatiites, as our calculations have shown.

Jordan<sup>33</sup> has argued, from seismic and heat-flow evidence, that the sub-continental lithosphere is composed of much more refractory mantle than the oceanic lithosphere, and that the thickness of this subcontinental 'tectosphere' may vary from as little as 100 km in young unstable regions to as much as 400 km beneath the older cratons. Oxburgh and Parmentier's model<sup>14</sup> provides a mechanism for the growth of this tectosphere through diapiric uprise of refractory Mg-rich lithosphere from the subducting slab. In this context greenstone belts could be considered as merely the surface expression of this process, where more active diapirs have managed to penetrate thin, immature, sub-continental tectosphere. Once a substantial tectosphere has developed beneath a continent it would, for reasons of density, viscosity and temperature, offer more resistance to penetration by such diapirs. Perhaps it is no coincidence then that most greenstone belts occur in regions which were geologically very young at the time the belts formed.



## Conclusions

This mechanism for the formation of Archaean ensialic greenstone belts may differ from that controlling the development of modern intra-oceanic marginal basins where, because of the more mature, cooler nature of the subducting lithosphere, the Benioff zone is steeply inclined. Toksöz and Bird<sup>34</sup> proposed that the down-dragging effect of the subducting slab induces convective circulation in the mantle wedge behind the volcanic arc, the broad upwardly convecting cell rupturing the lithosphere and causing back-arc spreading. Thus the effect may be different, but the strong link with subduction is present in both models. The original marginal basin model of Tarney *et al.*<sup>22</sup> was based on the Mesozoic ensialic *rocas verdes* complex in southern Chile where the amount of back-arc extension was very limited. When all aspects of greenstone belts are considered we feel that, when due allowance is made for the different Archaean thermal

regime and mantle structure, a marginal basin analogue is the model best able to accommodate the observed features of greenstone belts.

An important corollary is that extreme geothermal gradients implied for the Archaean mantle on the basis of the high liquidus temperatures of komatiitic lavas<sup>7</sup> are not necessarily correct. This is particularly important as far as eclogite stability in subduction zones is concerned because many Archaean crustal rocks have rare-earth element characteristics consistent with an origin through partial melting of eclogite<sup>35-37</sup>. Clearly also, caution must be exercised in deducing Archaean mantle compositions from high-magnesian komatiitic liquids; their compositions may merely reflect their local source regions.

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# Efficient translation of prokaryotic mRNAs in a eukaryotic cell-free system requires addition of a cap structure

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*In this and the accompanying paper we demonstrate that certain prokaryotic mRNAs, when modified at their 5'-termini with a cap structure, are translated in a eukaryotic cell-free protein synthesising system as efficiently as, or more efficiently than, eukaryotic mRNAs. Apparently, the prokaryotic mRNA contains all the information necessary for efficient recognition and initiation by eukaryotic translational components, except for the cap structure.*

MANY eukaryotic mRNAs are modified at their 5'-termini with a cap structure consisting of 7-methylguanosine in 5'-linkage with the first encoded base of an mRNA<sup>1-3</sup>. *In vitro* translation experiments with various eukaryotic cell-free protein synthesising systems indicate that the presence of the cap moiety is required for efficient translation of these messages<sup>4-18</sup>. Although the degree of cap-dependent translation varies among different mRNAs and depends on the *in vitro* translation system used<sup>8</sup> as well as on the particular conditions in which the translation reactions are carried out<sup>19,20</sup>, the results clearly suggest an important physiological role for the cap structure in the expression of these mRNAs.

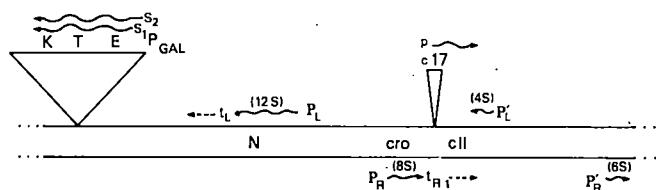
Various prokaryotic mRNAs (for example, RNA phage, T7 'early' RNA) have been translated in both mammalian and wheat-germ cell-free systems to produce accurate and functional protein products<sup>21-27</sup>. However, in these experiments it was necessary to use relatively high concentrations of the prokaryotic mRNAs to obtain detectable synthesis of the protein products. The translational efficiencies of these prokaryotic mRNAs were significantly lower than the corresponding efficiencies exhibited by equivalent amounts of most eukaryotic mRNAs (as judged by the amino acid incorporation per mol of mRNA added to the translation reaction). The prokaryotic mRNAs were, in fact, translated at levels equivalent to those obtained with eukaryotic mRNAs lacking cap structures.

In this and the following article<sup>28</sup> we examine the translation of several well-defined prokaryotic mRNAs in a wheat-germ cell-free system and, in particular, the effect of the enzymatic addition of a cap moiety on the translational efficiencies of these mRNAs. Our results indicate that these messages can be translated at relatively high efficiency in the wheat-germ extract and, analogous to the situation described for many eukaryotic mRNAs, this efficient translation is absolutely dependent on modification of the prokaryotic transcript with the cap structure. For each case examined, the capped prokaryotic mRNA was

found to translate as efficiently as, or more efficiently than, the equivalent amount (per mol) of capped rabbit haemoglobin (Hb) mRNA. Moreover, the cap-dependent translation exhibited by these mRNAs has allowed: (1) detection of an endogenous capping activity present in wheat-germ extracts, (2) comparison of the relative translational efficiencies exhibited by an mRNA encoding the same gene when located at different positions within a transcript, (3) demonstration that an mRNA which atypically initiates with a 5'-pyrimidine triphosphate can be cap-modified and translated, and (4) comparison of the translational efficiencies exhibited by two mRNAs which encode the same gene product but differ in their 5'-non-coding regions.

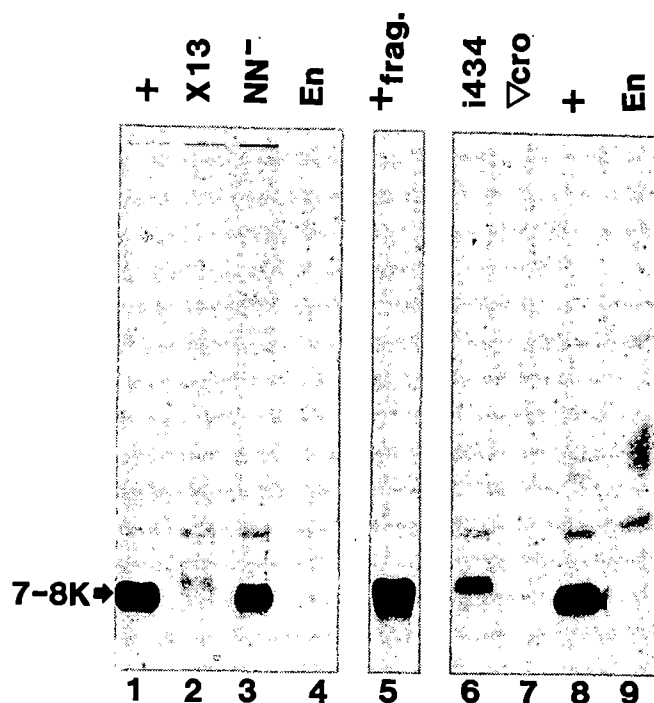
### Synthesis of the $\lambda$ *cro* gene product

Transcription of phage  $\lambda$  DNA with purified *Escherichia coli* RNA polymerase yields four major, well characterised RNA transcripts (Fig. 1)<sup>29-31</sup>. Two of these transcripts are relatively high molecular weight (MW) (>16S) polycistronic mRNAs which are known to code for a variety of phage functions. They initiate from the major early promoters of phage  $\lambda$ ,  $p_L$  and  $p_R$ , and extend leftward and rightward, respectively, from the  $\lambda$  immunity region (Fig. 1). In addition, two smaller transcripts are synthesised (4S and 6S RNAs), but these do not code for viral proteins. If the *in vitro* transcription reaction is carried out in the presence of the transcription termination protein factor, *rho*, then the  $p_L$  and  $p_R$  initiated mRNAs are terminated early at sites  $t_L$  and  $t_R$ , respectively, resulting in the production of discrete lower MW monocistronic mRNAs. A 12S RNA which encodes the  $\lambda N$  gene function is obtained from the left, and an 8S mRNA which encodes the  $\lambda$  *cro* gene product results from the right (Fig. 1)<sup>29-32</sup>.



**Fig. 1** Partial genetic map of bacteriophage  $\lambda$  showing the locations and relative sizes of the major RNA transcripts and some of the genes which they encode. In this study  $\lambda^+$  is actually  $\lambda b2$ , a derivative deleted for certain non-essential genes, thereby simplifying the *in vitro* RNA transcription pattern<sup>29</sup>. *c17* represents a small DNA insertion which occurs within the intercistronic boundary between the genes *cro* and *cII* and creates a new RNA polymerase promoter site (*p*) for RNA transcription<sup>32,44,45</sup>. The region of substitution of the entire galactose operon of *E. coli* (*gal* ETK) which occurs in the  $\lambda$  transducing phage,  $\lambda$  *pagal*8, is also indicated. All other designations are explained in the text.

RNA was prepared *in vitro* from a *rho* transcription reaction using  $\lambda$  DNA as template and then added to a wheat-germ cell-free translation system (Fig. 2). Addition of total RNA (that is, 4S, 6S, 8S and 12S RNA species) stimulated incorporation of <sup>35</sup>S-methionine into a single prominent polypeptide of ~7,500 daltons (Fig. 2-1). This protein was initially identified as the  $\lambda$  *cro* gene product by carrying out analogous translations on transcripts prepared identically from several well characterised  $\lambda$  mutants. The first,  $\lambda$  *x13*, carries a point mutation in  $p_R$  and is unable to initiate transcription of the 8S *cro* gene mRNA<sup>29</sup>. The second,  $\lambda$  *Δcro*, is deleted for the *cro* gene function. Although other RNAs are transcribed normally from the two mutants, these RNAs did not direct synthesis of the 7,500-dalton polypeptide (Fig. 2-2, 7). In contrast, translation of total RNA (all four species) prepared from a third mutant,  $\lambda$  *NN*<sup>-</sup>, which carries a double amber mutation in the *N* gene product (encoded by the 12S mRNA species)<sup>33</sup> did produce the 7,500-dalton polypeptide (Fig. 2-3). Thus, production of this polypeptide is apparently solely dependent on the presence of the 8S *cro* mRNA species. Identification was confirmed by preparing transcripts from a purified DNA restriction fragment which spanned



**Fig. 2** Autoradiograph of a 12.5% SDS-polyacrylamide gel analysis of the <sup>35</sup>S-methionine-labelled cell-free products synthesised in wheat-germ extracts in response to RNA transcribed *in vitro* from the indicated  $\lambda$  phage DNAs (see text for description of the various  $\lambda$  derivatives). Wheat-germ translations were carried out (as described below) in 50- $\mu$ l reactions using the following components: (1) ~1.0 pmol of 8S *cro* mRNA contained in ~0.8  $\mu$ g total RNA prepared from  $\lambda^+$  DNA; (2) ~0.8  $\mu$ g total RNA prepared from  $\lambda$  *x13* DNA; (3) 0.8  $\mu$ g total RNA prepared from  $\lambda$  *NN*<sup>-</sup> DNA; (4) no added RNA (endogenous); (5) ~1.2 pmol *cro* mRNA contained in ~150 ng total RNA prepared from a purified  $\lambda$  DNA restriction fragment (see below and text); (6) ~0.4  $\mu$ g total RNA prepared from  $\lambda$  *imm434* DNA; (7) ~0.4  $\mu$ g total RNA prepared from a  $\lambda$  derivative which is deleted for the *cro* gene,  $\lambda$  *bio7-20nutL44croΔ2*; (8) same as (1); (9) same as (4). RNA transcriptions were carried out as previously described<sup>31,32</sup> with the following modifications: reaction volume (0.5 ml), template DNA (2.5 pmol ml<sup>-1</sup>), *E. coli* RNA polymerase (40  $\mu$ g ml<sup>-1</sup>), purified *rho* factor (10  $\mu$ g ml<sup>-1</sup>). RNA synthesis was monitored and quantitated by incorporating [ $\alpha$ -<sup>32</sup>P]ATP (specific activity 10 mCi mmol<sup>-1</sup>) into a separate aliquot of the transcription reaction. Reactions were terminated by the addition of pancreatic DNase (25  $\mu$ g). After an additional 5 min incubation at 20 °C, the reaction mixture was made 0.1% in SDS, extracted with phenol, and precipitated twice with 2.5 volume of ethanol at -20 °C. The pellet was washed with 95% ethanol, dried and dissolved in H<sub>2</sub>O. For certain reactions (1, 5 and 8) the relative yields of 4S, 6S, 8S and 12S RNA species were determined by analysing the aliquot of <sup>32</sup>P-labelled RNA on a 3.5% polyacrylamide slab gel containing 8.0 M urea<sup>32,33,51</sup>. Wheat-germ cell-free translations were carried out essentially as described<sup>46</sup>. Reaction mixtures (50  $\mu$ l) contained micrococcal nuclease-treated wheat-germ extract (10  $\mu$ l), SAM (5  $\mu$ M), ATP (1 mM), GTP (0.4 mM), magnesium acetate (2.0 mM), spermidine (600  $\mu$ M, free base), creatine phosphate (8 mM), creatine phosphokinase (8  $\mu$ g ml<sup>-1</sup>; Sigma, 155 U per mg), KCl (85 mM), HEPES (25 mM, pH 7.6), amino acids minus methionine (25 mmol l<sup>-1</sup>, dithiothreitol (2 mM), <sup>35</sup>S-methionine (10-15  $\mu$ Ci at 400-800 Ci mmol<sup>-1</sup>, Amersham), and the indicated amounts of RNA. Protein synthesis was monitored by hot trichloroacetic acid precipitation as previously described<sup>46</sup>. Preparation and running of the samples on the 12.5% SDS-polyacrylamide slab gel have also been described<sup>46</sup>. After electrophoresis the gel was fluorographed for ~12 h at -70 °C (ref. 46). All values given represent final concentrations in the reaction mixture. The DNA restriction fragment used to prepare *cro* mRNA for translation reaction (5) was obtained from phage  $\lambda$  *r32*, a derivative which contains an IS2 insertion element within the *cro*-*cII* intercistronic boundary<sup>32</sup>. Restriction of this DNA with *Hind*III results in productions of a readily obtainable 1,600-base pair DNA fragment which spans the  $p_R$ -*cro* region (K. McKenney, unpublished data).

the *cro* cistron and contained only the  $p_R$  initiation site for RNA transcription. Translation of this RNA, which consists almost exclusively of the 8S *cro* mRNA, again results in production of the major 7,500-dalton polypeptide (Fig. 2-5).

Authentic *cro* protein has been obtained *in vivo* from  $\lambda$ -infected cells and is known to have a MW of 7,500 (ref. 34). Moreover, the nucleotide sequence of the region of the  $\lambda$  genome which encodes the 8S *cro* mRNA has been deter-

mined<sup>35</sup>, as has the amino acid sequence of the purified protein<sup>36</sup>. These data indicate that the 7,500-dalton *cro* polypeptide is a primary translation product and that the *cro* mRNA contains only one possible reading frame of codon triplets consistent with a protein of this size. Apparently, the 7,500-dalton protein being synthesised in the wheat-germ extract is the  $\lambda$ *cro* gene product. More recent studies (C. Queen, B.M.P. and M.R., unpublished results) indicate that translation of this same  $\lambda$  transcript in an *E. coli* S30 translation system results in the synthesis of the identical protein product.

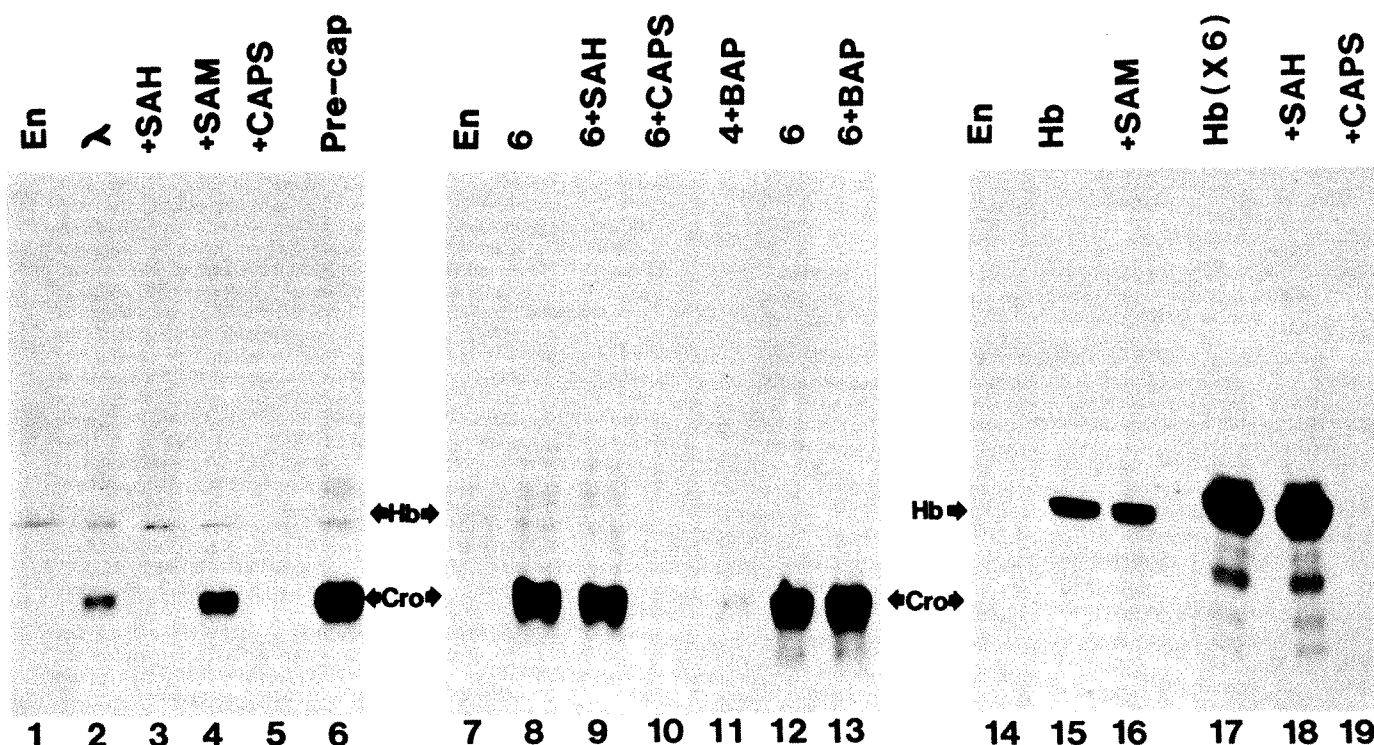
RNA transcribed from the  $\lambda$ x13 phage DNA template directed synthesis of a minor polypeptide of slightly higher MW than the  $\lambda$ *cro* protein (Fig. 2-2). This product did not seem to result from translation of any of the major  $\lambda$  mRNA species, but rather was accounted for by the following observations. The  $\lambda$ x13 phage is a replication-defective mutant and requires a helper phage for its growth. The helper phage (in this case the hetero-immune phage  $\lambda$ imm434) is subsequently separated from  $\lambda$ x13 by CsCl gradient centrifugation, although some cross-contamination between the two phages usually occurs. The  $\lambda$ 434 phage has an entirely different and somewhat larger *cro* gene than  $\lambda^+$  (ref. 37). Transcripts were prepared from purified  $\lambda$ 434 phage DNA and translated in the wheat-germ system (Fig. 2-6). Their translation resulted in the production of a single major polypeptide which had a gel mobility identical to the minor product observed from translation of the  $\lambda$ x13 mRNA (compare Fig. 2-2, 6). Apparently, the wheat-germ extract was able to translate the small amount of  $\lambda$ 434 *cro* mRNA which was transcribed with and contaminated the  $\lambda$ x13 mRNA preparation.

We emphasise that although the *cro* genes of  $\lambda^+$  and  $\lambda$ 434 are functional analogues, the nucleotide sequences of these two genes show little, if any, homology even in those regions involved in ribosome recognition and initiation of protein synthesis (ref. 37 and V. Pirrotta, unpublished data). Thus, the two *cro* genes actually represent independent examples of prokaryotic sequences which are being recognised and translated by the wheat-germ components.

### Synthesis of *cro* polypeptide is cap dependent

The cap structure is required for efficient translation of reovirus RNA<sup>4,38</sup>. This was demonstrated by monitoring the relative translational efficiencies exhibited by reovirus RNA which had been prepared *in vitro* in the presence or absence of *S*-adenosylmethionine (SAM). SAM acts as a methyl donor in the capping reaction and is required for the formation of a functional cap structure<sup>2</sup>. The viral RNA transcribed in the presence of SAM exhibited a significantly higher translational efficiency than RNA synthesised without added SAM. Moreover, it was observed that addition of *S*-adenosylhomocysteine (SAH) to the reovirus *in vitro* transcription system resulted in a sharp reduction in the ability to translate the reovirus RNA<sup>2,38</sup>. SAH is an analogue of SAM which competitively inhibits formation of the cap structure on the reovirus RNA.

We have used similar criteria to determine the extent of cap-dependent translation of  $\lambda$ *cro* mRNA in the wheat-germ cell-free system. As before, *cro* mRNA was prepared *in vitro* as



**Fig. 3** Composite autoradiograph of an SDS-polyacrylamide gel analysis (as in Fig. 2) showing the effects of the indicated manipulations on the cell-free synthesis in wheat-germ extracts of the  $\lambda$ *cro* polypeptide (1-13) and haemoglobin (Hb) protein (14-19).  $\lambda^+$  RNA was prepared *in vitro* (as described in Fig. 2) and identical aliquots of RNA (~0.5 pmol of 8S *cro* mRNA contained in ~0.4  $\mu$ g total RNA) were used for each translation reaction. Translations were carried out (as described in Fig. 2) except that *S*-adenosylmethionine was added only where indicated) in 50- $\mu$ l reactions using the following components: (1) no added RNA (endogenous); (2)  $\lambda$  RNA only; (3)  $\lambda$  RNA + *S*-adenosylhomocysteine (SAH, 500  $\mu$ M); (4)  $\lambda$  RNA + *S*-adenosylmethionine (SAM, 5  $\mu$ M); (5)  $\lambda$  RNA + m<sup>7</sup>GpppA (500  $\mu$ M); (6)  $\lambda$  RNA, which was initially cap-modified using the purified vaccinia capping enzymes (described below); (7) same as (1); (8) same as (6); (9) cap-modified  $\lambda$  RNA (as in 6) + SAH (500  $\mu$ M); (10) cap-modified  $\lambda$  RNA (as in 6) + m<sup>7</sup>GpppA (500  $\mu$ M); (11)  $\lambda$  RNA, which was initially treated with bacterial alkaline phosphatase (BAP, see below) + SAM (5  $\mu$ M); (12) cap-modified  $\lambda$  RNA (as in 6 and 8); (13) cap-modified  $\lambda$  RNA (as in 6), which was initially treated with BAP (as in 11); (14) same as (1) and (7); (15) Hb mRNA only (0.6 pmol); (16) Hb mRNA (0.6 pmol) + SAM (5  $\mu$ M); (17) Hb mRNA only (3.0 pmol); (18) Hb mRNA (3.0 pmol) + SAH (500  $\mu$ M); (19) Hb mRNA (3.0 pmol) + m<sup>7</sup>GpppA (500  $\mu$ M). RNA was cap-modified before translation using the purified vaccinia capping enzymes<sup>40</sup>. Reaction mixtures (10-40  $\mu$ l) contained Tris-HCl (50 mM, pH 7.5), MgCl<sub>2</sub> (1 mM), GTP (1-2 mM), dithiothreitol (5 mM), SAM (0.1 mM), RNA (~0.4  $\mu$ g) and enzyme extract<sup>40</sup>. After 20 min incubation at 37 °C, the reaction mixture was phenol extracted and precipitated twice with ethanol at -20 °C. The pellet was washed with 95% ethanol, dried, dissolved in H<sub>2</sub>O and then used directly for translation. Bacterial alkaline phosphatase treatment of RNA was carried out in reaction mixtures (0.1 ml) containing Tris-HCl (5 mM, pH 0.8), MgCl<sub>2</sub> (5 mM), RNA (0.4-1.0  $\mu$ g) and BAP (1.0  $\mu$ g, Sigma). After 20 min incubation at 37 °C, EDTA (10 mM) was added and the reaction mixture was phenol extracted and precipitated twice with ethanol at -20 °C. The pellet was washed with 95% ethanol, dried, dissolved in H<sub>2</sub>O and then used directly in the translation reaction.



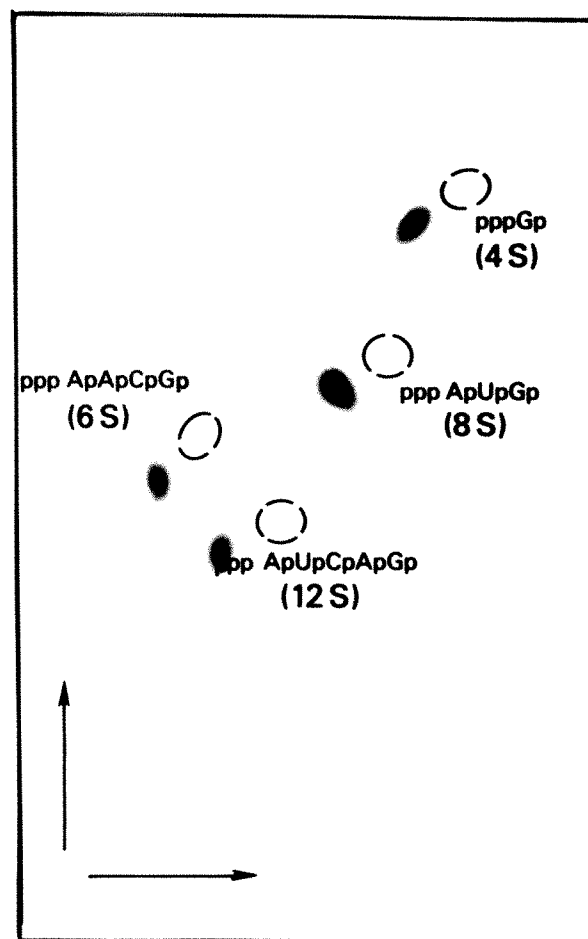
a primary 8S mRNA and subsequently translated in a standard wheat-germ translation reaction (Fig. 3). In this case, however, the translation reactions were carried out in the presence and absence of SAM and SAH. Addition of SAM resulted in markedly enhanced synthesis of the cro polypeptide (about threefold, compare Fig. 3-2, 4). In contrast, SAH addition (Fig. 3-3) completely and selectively abolished the synthesis of cro. These data indicate that translation of cro in the wheat-germ extract depends on an endogenous SAM-mediated (SAH-inhibited) methylation reaction. Analogous to the situation described above for reovirus RNA translation, this methylase activity is presumably that which is associated with the formation of a functional cap structure.

The observed effects are specific for an mRNA which requires endogenous cap modification for its translation. Similar addition of either SAM or SAH to translation reactions containing purified Hb mRNA had no effect on the synthesis of Hb protein (Fig. 3-15-18). As Hb mRNA already contains a 5'-cap structure, its translation is independent of the effects of SAM and SAH on the wheat-germ methylation activity. This is also true for the products which result from translation of the endogenous wheat-germ mRNAs present in the various reactions (compare Fig. 3-1-4). These RNAs, like Hb, already contain a cap structure and thus serve as direct internal controls in the translation reactions. Thus, the specificity of the effects observed with cro mRNA indicates that an endogenous capping activity present in the wheat-germ extract can appropriately modify the 5'-triphosphate terminus of the prokaryotic transcript and lead to its enhanced ability to be translated in the cell-free system. The presence of an enzymatic capping activity in the wheat-germ extract was recently confirmed (B. Moss, personal communication).

We have also directly demonstrated cap-dependent translation of the cro mRNA using enzymatic capping activity from vaccinia virions<sup>39,40</sup>. The enzymes were used to cap-modify the *in vitro* synthesised  $\lambda$  mRNA before translation in the wheat-germ system. The vaccinia enzymes add a cap structure to RNAs which contain either a 5'-di- or triphosphate terminus<sup>40</sup>. Capping reactions were initially carried out with [ $\alpha$ -<sup>32</sup>P]GTP so as to monitor and quantitate the extent of cap modification of the  $\lambda$  transcripts (see Fig. 4 legend). Analysis of these enzymatically capped RNAs by polyacrylamide gel electrophoresis indicated that each of the  $\lambda$  transcripts had incorporated <sup>32</sup>P label (not shown). More importantly, enzymatic digestion of this RNA with T<sub>1</sub> RNase and subsequent two-dimensional fingerprint analysis of the products demonstrated that the <sup>32</sup>P label was specifically transferred to four oligonucleotides which had mobilities consistent with the cap derivatives of the known 5'-terminal oligonucleotides of each of the four  $\lambda$  transcripts (Fig. 4). The specific activity of the products indicated that ~60% of the cro mRNA was cap-modified; enzymatic addition of the cap structure to the other three transcripts ranged between 40% and 60%.

$\lambda^+$  RNA, prepared *in vitro* and capped with the vaccinia enzyme, was added to the wheat-germ cell-free translation system. Translation of this RNA resulted in a >10-fold increase in production of cro protein as compared with translation of an identical amount of untreated transcript (compare Fig. 3-6, 2). Thus, direct cap modification of ~60% of the input prokaryotic message resulted in a dramatic increase in its ability to be translated in this eukaryotic system. Moreover, these pre-capped transcripts now continued to direct the synthesis of cro protein even in the presence of SAH (Fig. 3-9), analogous to the translation of Hb mRNA (Fig. 3-18). Cap modification with the vaccinia enzymes has eliminated the need for endogenous cap formation during the translation reaction.

The requirement for the integrity of the 5'-triphosphate group on the cro mRNA primary transcript was also demonstrated. Removal of the terminal phosphate residues by initial treatment of the RNA with bacterial alkaline phosphatase (BAP), followed by addition of this RNA to a translation reaction (+SAM) abolished synthesis of the cro polypeptide (Fig. 3-11).



**Fig. 4** Autoradiograph of a two-dimensional fingerprint (prepared as previously described)<sup>31,47,48</sup> showing the 5'-terminal RNase T<sub>1</sub> oligonucleotide products resulting from cap modification of the four  $\lambda$  RNA transcripts. Unlabelled  $\lambda^+$  RNA was prepared *in vitro* (as described in Fig. 2) and then cap-modified using the vaccinia enzymes, as described in Fig. 3 except that SAM was omitted from the reaction and [ $\alpha$ -<sup>32</sup>P]GTP (1-2 mCi, specific activity ~100 mCi  $\mu$ mol<sup>-1</sup>) was used to label specifically (by the cap-moiety) the 5'-triphosphate termini of the unlabelled transcripts. Horizontal dimension: electrophoresis on Cellolog in 8.0 M urea at pH 3.5. Vertical dimension: ascending thin-layer homochromatography on plates of DEAE-cellulose using homo-solvent B (refs 47, 48). The stippled circles show the approximate relative positions and nucleotide sequences of the unmodified 5'-terminal oligonucleotide products which are known to result from T<sub>1</sub> RNase digestion of the four  $\lambda$  transcripts (refs 49, 50, and unpublished data).

However, identical BAP treatment of  $\lambda$  RNA which had been pre-capped with the vaccinia enzymes had no effect on its ability to be translated (Fig. 3-13). Thus, the 5'-terminal triphosphate group was protected from phosphatase activity by the presence of the added cap structure.

Further evidence for the cap dependence of cro protein translation was obtained from experiments using m<sup>7</sup>G(5')pppA, a cap analogue which will inhibit the translation of capped mRNA<sup>14,20,41</sup>. Cap analogues seem to have little effect on cap-independent translation<sup>21</sup>. Addition of m<sup>7</sup>G(5')pppA to the translation reactions abolished the synthesis of both the Hb and cro polypeptides (Fig. 3-5, 19), as well as the synthesis of endogenous wheat-germ proteins (that is, background; compare Fig. 3-1, 5). Identical results were obtained whether cap modification occurred during the translation reaction (in response to added SAM, Fig. 3-5) or before translation using the vaccinia enzymes (Fig. 3-10).

Experiments similar to those described for the  $\lambda$ cro gene product were also carried out with the  $\lambda$ imm434 cro gene. Essentially identical results were obtained (data not shown). Again, we emphasise that these two prokaryotic mRNAs are quite different in primary structure, but exhibit similar cap dependence. In contrast to the results obtained with cro protein,

we were unable to detect synthesis of the  $\lambda$ N protein, the gene product encoded by the 12S mRNA species. Although cap modification of this RNA was achieved using the vaccinia capping enzymes (Fig. 4), no obvious translation product was observed. Structural characterisation of the 5'-terminal region of the 12S RNA suggests that the translation start point for the N protein may be relatively far (>200 nucleotide residues) from the 5'-triphosphate end (ref. 41 and K. McKenney, unpublished data). In addition, the 5'-non-coding region of this transcript is

known to be processed *in vivo* into several discrete RNA fragments<sup>43</sup>. Perhaps the length and/or structure of the 12S RNA leader region does not allow proper interaction between the 5'-terminal cap modification and the site for ribosome recognition and translation initiation of the N protein.

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# Efficient cap-dependent translation of polycistronic prokaryotic mRNAs is restricted to the first gene in the operon

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*Certain polycistronic prokaryotic mRNAs, when modified at their 5'-termini with a cap structure, are translated as efficiently as, or more efficiently than eukaryotic mRNAs in a eukaryotic cell-free protein synthesising system. However, in this case efficient cap-dependent translation is apparently restricted to the 5'-proximal coding sequence. Moreover, certain translational regulatory signals potentially used by these prokaryotic mRNAs to regulate their levels of expression seem to be recognised by the eukaryotic translational components. The evolutionary significance and practical implications of these results are discussed.*

IN the previous article<sup>1</sup> we demonstrated the cap-dependent translation of the monocistronic 8S  $\lambda$ cro mRNA in a wheat-germ cell-free protein synthesising system. These studies also allowed the detection of an endogenous capping activity present in the wheat-germ extract. Here, we extend these observations to other prokaryotic transcripts and, in particular, examine the cap-dependent translation of certain polycistronic mRNAs.

## Cap dependence in a polycistronic operon

Transcription of  $\lambda$  DNA in the absence of the protein termination factor *rho* results in the production of two high molecular weight (MW) polycistronic transcripts which are derived from the elongation of the 8S and 12S mRNAs<sup>2-5</sup> (see accompanying paper, Fig. 1). The *cro* gene now occurs at the 5'-end of a polycistronic transcript which is derived from the right operon of  $\lambda$  and encodes several other  $\lambda$  gene products. Translation of these high molecular weight mRNAs in the wheat-germ system again resulted in the synthesis of *cro* protein as the only major translation product (Fig. 1-1). Efficient translation of *cro* from the polycistronic message exhibited the identical dependence on cap modification to that observed for the 8S monocistronic mRNA (data not shown). The inability to detect protein synthesis from any of the cistrons located internally on the transcripts suggests that protein initiation sites positioned 'too far' from the cap structure may not be efficiently recognised by the eukaryotic translational components. Thus, it may be the relative proximity of the translation start site to the cap-modifiable 5'-triphosphate end of the transcript that is important for efficient *cro* protein translation. The following observations strongly support this contention.

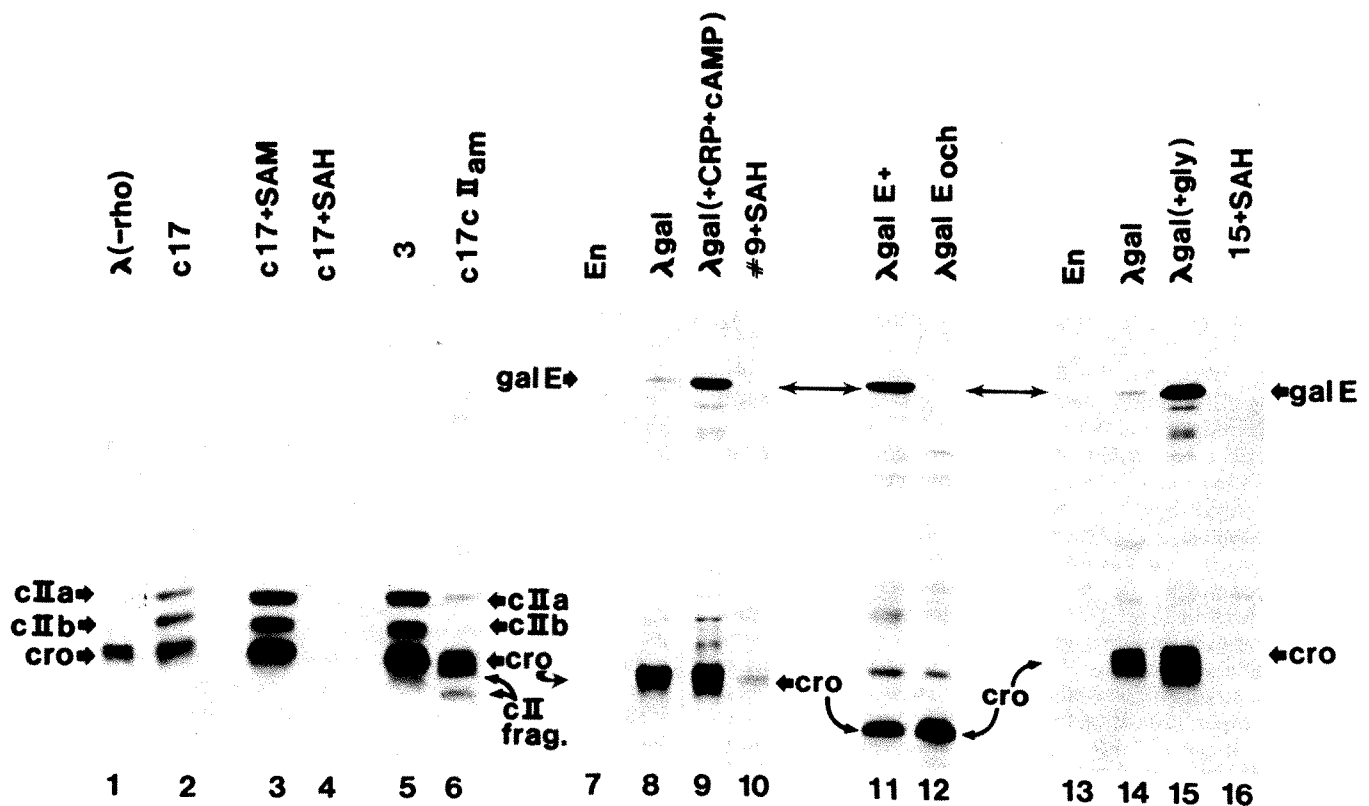
The *cII* gene of  $\lambda$  comprises the second cistron of the  $p_R$  promoted major rightward operon (see accompanying paper, Fig. 1). This gene is located distal to the *cro* gene and separated from it by an intercistronic regulatory region of ~120 base pairs<sup>5</sup>. A well characterised  $\lambda$  mutant,  $\lambda c17$ , contains a small DNA insertion within the intercistronic boundary between *cro* and *cII*, immediately preceding the presumptive ribosome binding region of *cII* (refs 5–7). This insertion creates a new promoter site for RNA polymerase and results in the transcription and functional expression of the *cII* coding region<sup>7</sup>. Transcription from this *c17* promoter initiates atypically with a pyrimidine triphosphate (CTP) and this 5'-triphosphate end occurs only 18 residues from the presumptive AUG initiation codon for *cII* (Fig. 3)<sup>5</sup>. Thus, *in vitro* transcription of the  $\lambda c17$  DNA template gives rise to one additional *c17* promoted transcript, which places the *cII* coding region in proximity to a new, cap-modifiable 5'-triphosphate end.

RNA transcripts were prepared *in vitro* using the  $\lambda c17$  DNA template and subsequently translated in the wheat-germ cell-free system (Fig. 1). In contrast to the results obtained using the  $\lambda^+$  DNA template, three major protein products were now obtained: the expected 7,500-dalton *cro* polypeptide and two additional proteins of ~12,000 and 14,000 daltons (Fig. 1–2). The single additional *c17* promoted transcript seemed to be responsible for the synthesis of both new polypeptides. This was confirmed when the same two protein products were obtained in

translation reactions carried out with RNA prepared from a purified DNA restriction fragment of  $\lambda c17$  (data not shown). This DNA fragment contains the *c17* promoter and the entire *cII* coding region, but lacks the major  $p_L$  and  $p_R$  promoters of  $\lambda$  (ref. 5).

Translation of both new polypeptides, as well as the *cro* protein, was dependent on cap modification of the  $\lambda c17$  mRNA. As with the translation of *cro* mRNA, synthesis of the two new polypeptides was markedly enhanced by S-adenosylmethionine (SAM) addition (Fig. 1–3) and sharply inhibited by S-adenosylhomocysteine (SAH) addition (Fig. 1–4) to the wheat-germ translation reaction. Moreover, cap modification before translation using the purified vaccinia enzymes again dramatically increased the production of both polypeptides (not shown).

Initial characterisation of the two new polypeptides was achieved by directing translation reactions with mRNA prepared from a derivative of  $\lambda c17$  which carries an amber point mutation within the *cII* gene ( $\lambda c17IIamber41$ ). Translation of the RNA surprisingly resulted in gel electrophoretic mobility shifts for both the 12,000 and 14,000 dalton polypeptides (Fig. 1–6). No change was detected for the *cro* polypeptide. As the amber mutation results from a single base-pair change<sup>9</sup>, both polypeptides must not only be derived from the *cII* coding region, but must also be translated in the same reading frame of codon triplets. Preliminary <sup>35</sup>S-methionine tryptic peptide mapping of these two *cII* proteins indicates that they do share



**Fig. 1** Composite autoradiograph of a gel analysis (as described in Fig. 2 of the accompanying paper) showing the <sup>35</sup>S-labelled products synthesised in wheat-germ extracts in response to RNA transcribed *in vitro* (see accompanying paper, Fig. 2) from various  $\lambda$  phage DNAs (see text for description of each  $\lambda$  derivative). Wheat-germ cell-free translations were carried out (as described in Fig. 3 of the accompanying paper) in 50- $\mu$ l reactions using the following components: (1)  $\lambda^+$  RNA (as in Fig. 3 of the accompanying paper except that *rho* factor was omitted from the transcription reaction); (2)  $\lambda c17$  RNA (containing ~0.6 pmol 8S *cro* mRNA in 0.5  $\mu$ g total RNA); (3)  $\lambda c17$  RNA (as in 2) + SAM (5  $\mu$ M); (4)  $\lambda c17$  RNA (as in 2) + SAH (500  $\mu$ M); (5) same as (3); (6)  $\lambda c17IIamber$  RNA (~0.4  $\mu$ g) + SAM (5  $\mu$ M); (7) no added RNA (endogenous); (8)  $\lambda ggal8$  RNA, prepared in the absence of *rho* factor; (9)  $\lambda ggal8$  RNA, prepared as in (8) except that CRP (40  $\mu$ g ml<sup>-1</sup>) and cyclic AMP (0.3 mM) were added to the transcription reaction, +SAM (5  $\mu$ M); (10) same RNA as in (9) + SAH (500  $\mu$ M); (11) same as (9); (12)  $\lambda ggalochreb4$  RNA, prepared with CRP (40  $\mu$ g ml<sup>-1</sup>) and cyclic AMP (0.3 mM) added to the transcription reaction; (13) same as (7); (14) same as (8); (15)  $\lambda ggal8$  RNA, prepared as in (8) and (14) except that glycerol (20%) was added to the transcription reaction; (16) same RNA as in (15) + SAH (500  $\mu$ M). All values given are final concentrations in the reaction mix. The two presumptive *cII* fragments indicated in (5) and (6) have not been shown to be derived from the *cIIamber41* mutation. The differences in relative intensity observed for the two *cII* fragments presumably result from the corresponding changes which occur in their respective methionine content. The minor band which remains at the *cIIa* position is an endogenous product (compare with Fig. 2-4 or 3-1 of the accompanying paper). The amounts of *gal E* specific mRNA added to translation reactions (8)–(16) were not determined; however, each translation reaction was directed with an identical aliquot of RNA obtained from each of the  $\lambda ggal8$  transcription reactions. Furthermore, these aliquots of RNA were estimated to approximate to those used in translation reactions 1–6.



common peptides and that the smaller protein consists mainly of a subset of the tryptic products contained in the larger protein (unpublished results). Moreover, the amber mutation seems to have resulted in a similar reduction in MW for each polypeptide (Fig. 1 see legend). If this is correct, then both the cII polypeptides must have the same (or nearly identical) carboxy termini and differ only (or predominantly) at their amino-terminal ends (by ~20–25 amino acid residues, Fig. 3).

An authentic 14,000 MW protein, which is derived from the cII coding region, has been identified from  $\lambda$ -infected cells<sup>10</sup>. The DNA sequence of the cII coding region predicts a protein of this MW<sup>9</sup>. Thus, the larger of the two cII proteins (cIIa, Fig. 1) synthesised in wheat-germ in response to the capped c17 transcript is likely to be this protein. The smaller 12,000-dalton polypeptide (cIIb) probably initiates translation 20–25 codon positions to the right of this site. The apparent size reduction resulting from chain termination observed for the two proteins would then be entirely consistent with the known nucleotide position of the amber mutation in the cII gene. Furthermore, a second AUG codon does occur at amino acid position 23 of the cIIa protein (position 84–87 in the c17 mRNA, Fig. 3). This AUG might serve as the initiator codon of cIIb. Ribosome binding studies support this contention (M.R. and B.M.P., in preparation). In separate reactions, translation components from either wheat-germ or *Escherichia coli* were used to form stable translation initiation complexes with the purified c17-promoted transcript. Both the 60S prokaryotic ribosome and the 70S eukaryotic ribosome were found to bind specifically and protect (from ribonuclease digestion) two separate and identical regions of this RNA. Each protected site spanned about 30–40 nucleotide residues and centred around one of the appropriate AUG codons. It is not known whether these two overlapping sites for protein synthesis have any functional significance for the bacteriophage. However, recent studies (C. Queen, B.M.P. and M.R.) indicate that two proteins of identical electrophoretic mobilities to those made in wheat-germ can be synthesised in an *E. coli* S30 translation system using the same *in vitro* prepared  $\lambda$  transcripts. Thus, the wheat-germ translational components may be recognising a translational regulatory mechanism used by  $\lambda$  to produce two different polypeptides from the same coding region.

Regardless of the possible functional implications of the two overlapping cII proteins, their translational in the wheat-germ system required cap modification of the c17-initiated mRNA. As we described earlier, the sequences encoding the cII regulatory region also occur on the  $p_R$ -initiated polycistronic transcript which was synthesised from the  $\lambda^+$  DNA template. In this case, cII is the second cistron of the operon, located ~330 nucleotide residues away from the cap-modified 5'-end. Translation of the  $p_R$ -promoted mRNA did not result in detectable synthesis of either cII protein (Fig. 1-1). Only on introduction of a new transcription start site (through the c17 promoter), which placed a cap-modifiable 5'-triphosphate terminus in the proximity of the cII coding region, was efficient translation of cII obtained. Moreover, cap modification of the initiating pyrimidine nucleotide, CTP, of the c17 mRNA resulted in efficient translation from two different sites, respectively, 18 and 84 residues from the cap structure. The larger protein (initiated closer to the cap) was always obtained in somewhat higher yield (~60%) than the smaller cII protein (~40%, normalised for methionine content). We do not know whether the two start sites represent functionally independent regions for translation initiation or whether their function is related. The mechanism which leads to the cap-dependent synthesis of the two cII proteins is being studied.

### Cap-dependent *gal E* translation

The *gal* operon of *E. coli* consists of three structural genes, *E* (epimerase), *T* (transferase) and *K* (kinase), which are transcribed into a single polycistronic mRNA from a promoter region which has been well characterised<sup>11–13</sup> (see accompanying paper, Fig. 1). *In vitro*, transcription of *gal* DNA can occur

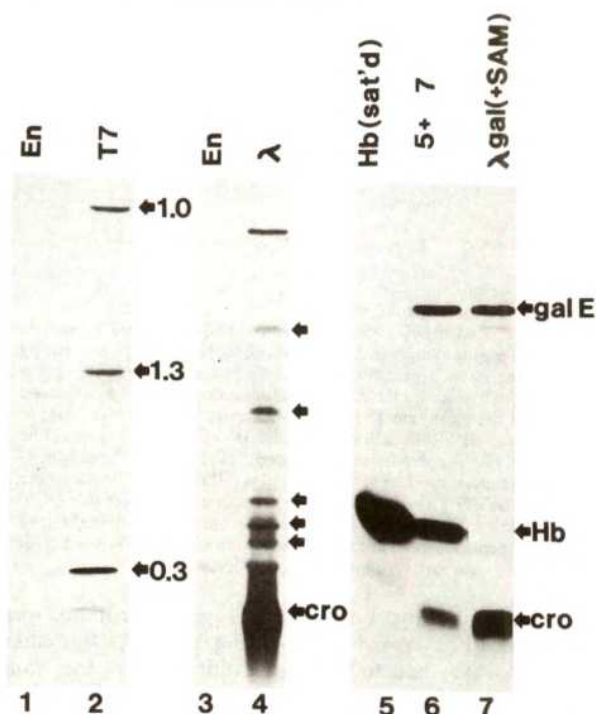
from either of two distinct sites which are known to be 5 base pairs apart within the *gal* regulatory region (start sites *S*<sub>1</sub> and *S*<sub>2</sub>, Fig. 3)<sup>14</sup>. Transcription from start site *S*<sub>1</sub> requires the presence of cyclic AMP receptor protein (CRP) and cyclic AMP in the transcription reaction<sup>14</sup>. In these conditions, no transcription initiates from start site *S*<sub>2</sub>. In the absence of CRP and cyclic AMP, *S*<sub>1</sub> transcription is abolished and only low-level *S*<sub>2</sub> transcription occurs (~20-fold lower than the CRP-cyclic AMP-dependent *S*<sub>1</sub> transcription). However, CRP-cyclic AMP-independent *S*<sub>2</sub> transcription can be stimulated (~8–10-fold) by the addition of glycerol to the *in vitro* reaction<sup>15</sup>. Thus, using the appropriate *in vitro* transcription conditions, two distinct transcripts of the *gal* operon can be obtained which differ only in that one (CRP-cyclic AMP independent) has five additional nucleotide residues at its 5'-end compared with the other (CRP-cyclic AMP-dependent transcript) (Fig. 3).

RNA was prepared *in vitro* using DNA obtained from a  $\lambda$  transducing phage,  $\lambda_{pgal8}$ , which carries the entire *gal* operon within its genome (see accompanying paper, Fig. 1). These transcripts consist of the various  $\lambda$  mRNAs (described earlier), as well as the additional *gal*-derived polycistronic mRNA. Transcriptions were initially carried out either in the presence or absence of CRP-cyclic AMP and this RNA was then translated in the wheat-germ cell-free system (+SAM, as described in Fig. 1). RNA, prepared in the presence of CRP-cyclic AMP, directed synthesis of a new major protein product (~38,000 daltons, Fig. 1-9). Only minor amounts of this same protein were produced in a translation reaction carried out identically on RNA made in the absence of CRP-cyclic AMP (Fig. 1-8). Transcription of the *gal* operon, specifically induced by CRP-cyclic AMP, resulted in the corresponding translation of the 38,000-dalton protein. Cro protein was also made in these reactions (in response to the  $\lambda_{pR}$ -initiated mRNA); however, its synthesis was not affected by the presence or absence of CRP-cyclic AMP (compare Fig. 1-8 and 1-9). The CRP-cyclic AMP-dependent protein product is presumably *gal* epimerase (*E*), the 38,000-dalton protein encoded by the first cistron of the *gal* operon. Identification of the *gal E* product was confirmed by the translation of transcripts prepared from a mutant of  $\lambda_{pgal8}$ , which carries an ochre, chain-terminating mutation early within the *E* gene (S. Adhya, unpublished). These transcripts specifically failed to direct synthesis of the 38,000-dalton protein, whereas cro protein was produced normally (Fig. 1-12).

Analogous to the results obtained with both *cro* and cII, *gal E* translation in the wheat-germ system was also completely dependent on cap modification of the *S*<sub>1</sub>-initiated *gal* transcript. Addition of SAH to the translation reaction abolished synthesis of the *gal E* protein (as well as cro protein being synthesised in the same reaction, Fig. 1-10). In addition, *gal E* synthesis was markedly enhanced by cap modification of the RNA with the purified vaccinia enzymes before its translation. Although the *gal* mRNA encodes three gene products (*E*, *T* and *K*), again only the 5'-proximal *E* gene seemed to be translated efficiently. The AUG initiation codon for *gal* epimerase occurs only 27 residues from the 5'-cap-modified triphosphate end of the *S*<sub>1</sub>-initiated *gal* transcript<sup>15</sup>. It is difficult to compare the relative cap-dependent translation efficiencies of *gal E* and *cro* as the number of methionine residues in epimerase is not known. However, the *gal* promoter, even when stimulated 20-fold by CRP-cyclic AMP, is still less efficient *in vitro* than the  $\lambda_{pR}$  promoter. Thus, the apparently weaker translational response observed for *gal E* (compared to *cro*) results, at least in part, from the fact that there is less *gal* RNA produced in these transcription reactions than there is *cro* mRNA.

Several minor protein bands also seem to be synthesised in the wheat-germ extract in response to the CRP-cyclic AMP dependent *gal* transcript (Fig. 1-9). The appearance of these products was also sensitive both to the effects of SAH (Fig. 1-10) and to the introduction of the *gal E* ochre chain-terminating mutation (Fig. 1-12). These minor products probably result from premature termination of either *gal E* transcripts or translation products and were not further characterised.





**Fig. 2** Composite autoradiograph of a gel analysis showing the  $^{35}\text{S}$ -methionine-labelled products synthesised in wheat-germ extracts in response to RNA transcribed *in vitro* from the indicated phage DNAs (1–4) and a competition experiment comparing the relative translational efficiencies of *cro*, *gal E* and Hb mRNA (5)–(7), see below and text for details. Translation reactions 1–4 were carried out (as in Fig. 1) using the following components: (1) no added RNA (endogenous); (2) T7 early RNA (2.0  $\mu\text{g}$  total RNA containing  $\sim 1.0$  pmol of polycistronic T7 mRNA); (3) same as (1); (4)  $\lambda^+$  RNA, transcribed in the absence of *rho* factor (2.0  $\mu\text{g}$  total RNA containing  $\sim 1.0$  pmol of the  $p_R$ -initiated polycistronic *cro* mRNA). Translation reactions (5)–(7) were carried out (+5  $\mu\text{M}$  SAM) in reaction mixtures (50  $\mu\text{l}$ ) containing only 50% the wheat-germ extract (5  $\mu\text{l}$ ) as was used in all previous reactions (see Fig. 2 of the accompanying paper). This 'diluted' cell-free system was found to be saturated for translation of 3.0 pmol of Hb mRNA. Translations were carried out with the following components: (5) Hb mRNA only (3.0 pmol); (6) Hb mRNA (3.0 pmol) +  $\lambda\text{gal}18$  RNA (containing  $\sim 0.5$  pmol of *cro* mRNA and even less (but an undetermined amount) of *gal E* mRNA (see text)); (7)  $\lambda\text{gal}18$  RNA only (as in (6)). RNA was transcribed from T7 DNA as previously described<sup>29</sup>, with the following modifications: reaction volume (1.0 ml); T7 DNA (100  $\mu\text{g ml}^{-1}$ ); RNA polymerase (50  $\mu\text{g ml}^{-1}$ ). The T7 RNA directed translation products were identified by comparing their electrophoretic mobilities with those T7 products identified by Anderson *et al.*<sup>17</sup>. Apparent  $\lambda$  translation products ( $\rightarrow$ ), other than *cro* polypeptide, were not identified. Gel analyses of translation reactions (1)–(4) were autoradiographed for  $\sim 24$  h (twice the exposure time of all other gel analyses).

### *gal E* from another mRNA

Experiments similar to those described above were also carried out with RNA transcribed in the presence of glycerol using the  $\lambda\text{gal}18$  DNA template. Glycerol affects the level of RNA synthesis occurring from both the  $\lambda$  and *gal* promoters<sup>15</sup>. CRP-cyclic AMP-independent *gal* transcription (from start site  $S_2$ , Fig. 3) is increased 8–10-fold, whereas transcription of the  $\lambda p_R$  operon is enhanced only twofold<sup>15</sup>.

RNA was purified from a glycerol-stimulated transcription reaction and translated in the standard wheat-germ cell-free system. Again, two major protein products were synthesised: the 7,500-dalton *cro* protein and the 38,000-dalton *gal E* protein (Fig. 1–15). The translation system seems to have responded to the glycerol-enhanced levels of both *cro* and *gal* RNA (compare with Fig. 1–14). Using the same criteria as before, these transcripts were also shown to require cap modification for their translation in the wheat-germ extract (see, for example, Fig. 1–16).

*gal* mRNA stimulated with glycerol initiates at start site  $S_2$  and has a 5'-non-coding region 32 nucleotides long<sup>14</sup>. This places the cap structure 5 nucleotides farther from the presumptive *gal E* initiation site than occurs on the CRP-cyclic AMP-dependent,  $S_1$ -initiated mRNA. Although both

*gal* mRNAs are dependent on cap modification for their efficient translation in the wheat-germ system, these two messages exhibited different translation efficiencies. Approximately twice the amount of *gal* mRNA is made in the CRP-cyclic AMP-dependent transcription reaction compared with the glycerol-stimulated reaction<sup>15</sup>. However, the more abundant CRP-cyclic AMP-dependent  $S_1$  mRNA directed synthesis of less ( $\sim 50\%$ ) *gal E* protein than did the glycerol-induced  $S_2$  mRNA (compare Fig. 1–9, 15). Assuming that cap modification occurs to similar extents on the two different mRNAs, then the  $S_2$ -promoted mRNA is being translated 4–5-fold more efficiently than the  $S_1$ -promoted mRNA.

It was surprising that the *gal* mRNA was translated more efficiently when the cap moiety occurred 5 nucleotides farther from the translation start point. Presumably, the translational differences exhibited by the two *gal* mRNAs reflect the corresponding differences in their 5'-terminal structures (Fig. 3). The primary structural differences may affect the higher order structures (secondary and tertiary) exhibited by the non-coding leader regions of these two RNAs. Thus, the overall structure of the regions involved in recognition and initiation of *gal E* translation may be different in the two *gal* messages and thereby account for the differences in their translation efficiencies. As the translation system is derived from wheat-germ and requires cap modification of the *gal* mRNAs, the observed effects may be specific for translation in this particular system and therefore somewhat artefactual.

Alternatively, it is possible that the wheat-germ system is recognising and responding to a translational regulatory mechanism normally used to regulate *gal* enzyme levels in bacteria. The *gal* operon is known to be coordinately expressed when cells are metabolising galactose<sup>11</sup>. In the absence of galactose, however, *gal E* expression is constitutively required for bacterial cell-wall synthesis<sup>11</sup>. It has been postulated that the dual promoter system of *gal* regulates these different requirements for *gal* enzyme levels. Recent experiments (S. Adhya, personal communication) further indicate that, *in vivo* during CRP-cyclic AMP-independent *gal* expression, *gal E* activity is fourfold higher (whereas *gal K* activity is lower) than during CRP-cyclic AMP-dependent *gal* expression. If (as *in vitro*) the CRP-cyclic AMP-independent promoter (from  $S_2$ ) is less efficient at *gal* transcription than the CRP-cyclic AMP-dependent promoter (from  $S_1$ ), then perhaps the higher levels of *gal E* activity observed in the absence of CRP-cyclic AMP can be accounted for by more efficient translation of the  $S_2$ -initiated mRNA. If this is the case, then the wheat-germ components may not only be efficiently translating the cap-modified prokaryotic mRNA, but may also be responding to other translational regulatory signals encoded within these transcripts.

### Efficiency of capped and uncapped prokaryotic mRNAs

Identical amounts ( $\sim 0.5$  pmol) of *cro* mRNA were added to each of the translation reactions depicted in Fig. 3 of the accompanying paper. However, the amounts of *cro* protein synthesised in each of these reactions differed, due to the different relative extents to which the *cro* mRNA had been cap-modified in the various reactions. Approximately 60% of the *cro* mRNA (0.3 pmol) was shown to be cap-modified using the vaccinia enzyme. Translation of this RNA resulted in a  $>10$ -fold enhancement in *cro* protein synthesis, as compared with direct translation of the  $\lambda$  transcript (see accompanying paper<sup>1</sup>, Fig. 3–2, Fig. 3–6). If the translational activity exhibited by the *cro* mRNA is proportional to the number of functionally capped transcripts, then the amount of *cro* product obtained from direct translation of the  $\lambda$  RNA represents cap modification of  $<6\%$  of the input RNA (that is,  $<0.03$  pmol). Addition of SAM to the translation system was shown to stimulate *cro* translation about threefold. Thus, the wheat-germ extract seems to be capable (in the presence of SAM) of cap modifying about 15–20% of the input *cro* mRNA (that is,  $\sim 0.1$  pmol) and translating it with relatively high efficiency.



Our ability to detect readily the synthesis of the prokaryotic gene products in response to such small quantities of RNA is unprecedented. Other workers have reported accurate translation of several prokaryotic RNAs in various eukaryotic translation systems<sup>17-23</sup>. The quantities of RNA used in these studies and (where data are available) the relative yields of protein synthesis obtained suggest significantly lower translation efficiencies than those reported here for the cap-modified prokaryotic mRNAs. We were also able to demonstrate relatively low-level, cap-independent translation for several prokaryotic mRNAs. Transcripts were prepared using bacteriophage T7 DNA as template (see Fig. 2 legend). Anderson *et al.*<sup>17</sup> have demonstrated that T7 RNA is accurately translated in both a wheat-germ and a mammalian cell-free translation system. Furthermore, translation of this RNA was shown to be completely independent of cap modification. Although our results agree with theirs, we find that the T7 RNAs are translated relatively poorly when compared with the cap-modified  $\lambda$ , *gal*, or haemoglobin (Hb) mRNAs. Translation of 10-fold more T7 RNA (~1.0 pmol, Fig. 2-2) than capped *cro* mRNA (0.1 pmol; see accompanying paper, Fig. 3-4) resulted in the synthesis of significantly less T7 protein. It is difficult to compare precisely the synthesis of the T7 proteins with that of *cro*, as the number of methionine residues contained in the T7 protein is not known. Even if we assume that each of the T7 proteins contains only two methionine residues (as does *cro*), then their efficiencies of translation were 50-100 times lower than for *cro*.

Results similar to those observed with the T7 proteins were also obtained for several  $\lambda$  proteins. Addition of more polycistronic  $\lambda$  mRNA to the wheat-germ translation system and longer autoradiographic exposure times allowed detection of several other  $\lambda$  specific protein products (Fig. 2-4). Some of these polypeptides have MWs consistent with proteins known to be encoded by the polycistronic  $\lambda$  RNAs. These products have not been further characterised. Thus, the wheat-germ translation components can recognise and initiate translation of these other prokaryotic gene products, albeit at lower efficiency. Comparison (within the same reaction, Fig. 2-4) of the low-level synthesis of these proteins with the level of cap-dependent synthesis of *cro*, clearly demonstrates the importance of the cap structure for the efficient translation of the first gene in the polycistronic transcript.

### Efficiency of *cro* and Hb

The translation efficiency of the cap-modified prokaryotic mRNAs was also compared with that of purified Hb mRNA. Both *cro* protein and Hb protein have identical methionine contents and thus their abilities to be translated can be compared directly. In separate translation reactions, cap-modified *cro* RNA (~0.3 pmol) was shown repeatedly to direct higher levels of protein synthesis than did twice the amount of Hb RNA (~0.6 pmol; see accompanying paper, Fig. 3-6, Fig. 3-15). The capped prokaryotic transcript consistently translated about fivefold more efficiently than did Hb RNA in the wheat-germ extract. Experiments similar to these (unpublished) were also carried out using the standard reticulocyte lysate translation system. Again, *cro* RNA, cap-modified before translation with the vaccinia enzymes, was translated more efficiently than Hb RNA.

The remarkable ability of the capped, prokaryotic mRNA to be efficiently translated was even more pronounced when compared directly with Hb mRNA in the same translation reaction. Limiting conditions were found (legend to Fig. 2) in which the translation system was apparently saturated for the translation of Hb RNA. Using these conditions, RNA which had been prepared from a *λgal8* DNA template (in the presence of CRP-cyclic AMP) was added to the translation reaction (+SAM) simultaneously with saturating amounts of Hb mRNA (3.0 pmol). Although Hb RNA was present in about sixfold greater excess than total *cro* mRNA (~0.5 pmol), we observed a sharp reduction in the ability of the system to synthesise Hb

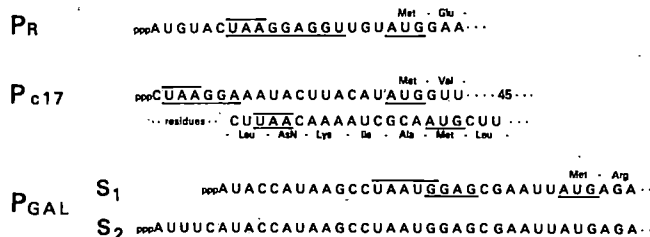


Fig. 3 The 5'-terminal nucleotide sequences of the RNA transcripts which encode the *Acro* protein (from promoter *p<sub>R</sub>*)<sup>30,31</sup>, the *λcII* protein(s) (from promoter *c17*)<sup>5</sup>, and the *E. coli gal* E protein (from the dual promoters *pgal*)<sup>13,16</sup>. The presumptive translation start sites for each polypeptide being translated in the wheat-germ cell-free extract is indicated AUG. The region of the RNA which precedes the AUG initiation codon for each protein, and which exhibits the most extensive complementarity to either the 3'-end of the prokaryotic 16S rRNA component (underlined) or the 3'-end of the eukaryotic 18S rRNA component (overscored) is also shown. The presumptive protein initiation sites for both *cII* proteins (*cIIa* and *cIIb*, see text) are indicated on the *c17* promoted transcript. The smaller polypeptide (*cIIb*) starts 22 amino acid residues (66 nucleotides) downstream from the start of the larger polypeptide (*cIIa*). The two transcription start sites (S<sub>1</sub> and S<sub>2</sub>) for *gal* mRNA synthesis are also shown<sup>14</sup>.

protein. Concomitantly, both *cro* and *gal* E proteins were readily produced (compare Fig. 2-5, Fig. 2-6). As the added prokaryotic mRNA had to be cap-modified during the translation reaction (in the presence of SAM), only 15-20% of the input *cro* message (that is, 0.1 pmol) accounted for the *cro* protein synthesised. Thus, the translation products shown in Fig. 2-6 actually represent those resulting from translation of an effective 30-fold difference in the amounts of capped *cro* and Hb mRNA (an even greater difference for the *gal* mRNA). The cap-modified prokaryotic message is clearly very efficient at competing for limiting eukaryotic translational components.

### Other implications

We have demonstrated the importance of the cap structure for obtaining efficient translation of certain prokaryotic mRNAs in a eukaryotic cell-free system. It is remarkable that cap modification of these transcripts is sufficient to result in their expression at levels which simulate (and actually surpass) those normally exhibited by many capped eukaryotic mRNAs. Our results indicate that, except for the cap modification, these prokaryotic mRNAs must contain all the essential structural information required for proper recognition and efficient synthesis by the eukaryotic translational components. Apparently strong evolutionary constraints have been placed on that portion of the mRNA structure that initiates translation, as well as on those regions of the ribosome which correspondingly interact with this RNA structure.

As the prokaryotic message does not require the cap structure for its normal expression in bacteria, our findings suggest that the steric requirements necessary for normal cap function are not too stringent. The positioning of the 5'-capped end of an RNA, however, is clearly subject to some structural limitations. Cap-dependent translation of the *λcII* gene was only obtained by positioning the cap structure nearer the *cII* coding region (through the *c17* mutation). The same *cII* coding information positioned ~330 residues from the cap structure was not translated effectively. RNA structural effects were also implicated in the apparent differential translation observed for the *gal* E protein from the two *gal* mRNAs. These observations suggest that prokaryotic and eukaryotic mRNAs may contain similar higher order structural requirements in those parts of the RNA molecule which are necessary for ribosome recognition and the initiation of protein synthesis.

Other signal sequences occurring on mRNA have been implicated in translational regulation<sup>24-28</sup>. The prokaryotic mRNA contains a region (of variable length) just preceding the AUG initiation codon (at a reasonably conserved distance), which is complementary to the 3'-terminus of the bacterial 16S rRNA components<sup>24-28</sup>. There is evidence that this region and its interaction with the 16S rRNA are important in the



translation of at least some prokaryotic mRNAs. It has also been suggested that the 5'-non-coding region of the eukaryotic mRNA may contain a similar type of recognition signal which correspondingly interacts with the 3'-end of the eukaryotic 18S rRNA component<sup>25</sup>. However, the heterogeneity in size and nucleotide sequence of the 5'-non-coding region of the eukaryotic mRNAs suggest that, except for the AUG initiation codon, it is difficult to find a conserved region which exhibits the necessary complementarity<sup>26,27</sup>.

The prokaryotic mRNAs used in our studies also exhibit little complementarity (except for the AUG initiation codon) to the 3'-end of the eukaryotic 18S rRNA. The  $\lambda$ cro and cII mRNAs, for example, can form only 3 A · U base pairs with the eukaryotic rRNA component. In contrast, these same mRNAs exhibit relatively extensive complementarities to the bacterial 16S rRNA component (Fig. 3). The ability of the prokaryotic mRNAs to be translated so efficiently by the wheat-germ system suggests that the necessity for the complementarity feature has been markedly diminished in the eukaryotic translation system. Although it is possible that an alternative recognition signal(s) has evolved in the eukaryotic mRNA, this signal should not be present in the prokaryotic message. As cap modification is the

only alteration required for efficient translation of the prokaryotic mRNA, perhaps the cap moiety now fulfills this recognition function.

The ability of the cap-modified prokaryotic message to compete efficiently with even saturating amounts of a eukaryotic mRNA (Fig. 2-6) suggests the possibility of obtaining high-level, functional expression of prokaryotic genes on their introduction into eukaryotic cells. Of course, appropriate transcriptional and post-transcriptional modification of the prokaryotic sequences would be required. Furthermore, it may be possible to increase the level of synthesis of essentially any gene product within a eukaryotic cell. Those prokaryotic sequences which contain highly efficient recognition signals for eukaryotic translational components (for example, 5'-non-coding region of the  $\lambda$ cro gene) could be fused (using available genetic engineering techniques) to eukaryotic sequences encoding protein structural information. Experiments of this nature are under way.

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## letters

### A kinematic model for SS433

THE bizarre object SS433, first noted because of its H $\alpha$  emission<sup>1</sup> and most recently because it is the optical counterpart of a variable radio and X-ray source<sup>2-4</sup>, exhibits an extraordinary optical spectrum<sup>5</sup>. Three sets of emission lines of hydrogen and helium appear: one is near zero radial velocity (less than 180 km s<sup>-1</sup>), and the other two show large and variable shifts to the blue and red. These shifts are almost unquestionably due to the Doppler effect, as multiple lines with constant  $z \equiv \Delta\lambda/\lambda$  have been identified in both the red and blue systems<sup>6-8</sup>. The observed range of shifts<sup>5,8</sup> corresponds to velocities from 0 to -30,000 km s<sup>-1</sup> in the blueshift system, and 11,000 to 48,000 km s<sup>-1</sup> in the redshift system, with the two sets of lines moving ~180° out of phase with each other. At present the red- and blueshift variations seem periodic, with a period of about 164 d, based on the observation of parts of three cycles<sup>7,8</sup>.

The emitting regions giving rise to the variable wavelength systems cannot be objects in mutual orbital revolution, for with a 164-d period and velocities of 0.15 *c*, keplerian orbits would have light-crossing times of the order of weeks, and the blue- and red-shifted emission lines could not continue to have simultaneous oppositions. The emission, therefore, must come from regions relatively close to a central object. A lower limit on the

distance to SS433, based on interstellar line strengths and velocities<sup>5</sup>, is about 3.5 kpc. The low velocity of the zero-redshift system suggests that the object is probably galactic, although certain *ad hoc* extragalactic hypotheses cannot be ruled out.

Milgrom<sup>9</sup>, independently of the present work, has also noted that the variations in redshift could be periodic and sinusoidal, and he considered several possible models to account for the variable velocity systems, including what we believe to be the correct one. In our judgement, Milgrom showed remarkable insight in interpreting the few data<sup>5</sup> then available to him. Now the much expanded observations<sup>8</sup> are sufficient to narrow the possibilities substantially.

We suggest here that the radiation with variable Doppler shift is emitted by hot matter ejected by the central object at high but nearly constant velocity in oppositely directed narrow streams, possibly along a magnetic axis. Rotation of the beam axis provides the observed radial velocity variations. This model is thus a modification of that proposed for SS433 by Fabian and Rees<sup>10</sup>, where the beam velocity is modulated to create these variations. In our interpretation for SS433, as the matter reaches a critical distance from the central object, it cools sufficiently to recombine and give rise to the observed emission. The stationary system of lines may originate from low velocity gas in the vicinity of the object or possibly from matter ejected at an earlier

time and later re-excited. The radiating matter could also be infalling along a collimated axis, although the source of such matter then requires special explanation. Conversely, the matter can be ejected (or infalling) isotropically from (or onto) the object, and be illuminated by two ionising radiation beams from the centre. The problem of accounting for the copious source of material in the latter case is far from trivial. We are concerned here not with a specific mechanism, but rather with a kinematic model that accounts for the current observations; it is very similar to model 3 suggested earlier by Milgrom<sup>9</sup>. Our model includes specific predictions regarding the behaviour of the variable velocity components during the as yet unobserved portions of the 164-d period. In particular, the behaviour in the summer of 1979 is predicted to show correlated variations with much lower amplitude than previously observed. This asymmetric behaviour, if observed, will provide some guide to the range of astrophysical mechanisms involved, and also yield direct observational evidence that the system period is actually 164 d, and not an integer multiple thereof.

We assume that the beam axis rotates with angular velocity  $\omega = 2\pi/164$  d. Although the cause of this rotation is unimportant to our simple kinematic description, note that the extremely high ratio of total system luminosity to the rotational energy of a compact star with a 164-d period implies that simple stellar rotation locked to the beams may not be the basic underlying system clock; precession is a possible alternative<sup>11</sup>. Let the line-of-sight lie along a unit vector  $\hat{n}$ , inclined at angle  $i$  to the beam rotation axis. As the beam axis rotates, the jets of flowing matter, orientated at angle  $\theta$  to the rotation axis, describe a conical motion about this axis, with period 164 d. Let the matter stream in opposite directions at speed  $v$  (in units where  $c = 1$ ), and let  $\mathbf{V}$  be the velocity vector of the stream in the same hemisphere as  $\hat{n}$ . The direction cosine,  $l$ , of the angle between  $\mathbf{V}$  and  $\hat{n}$  is

$$l = \frac{\mathbf{V}}{v} \cdot \hat{n} = \sin i \sin \theta \cos \omega t + \cos i \cos \theta$$

$$\equiv a \cos \omega t + b \quad (1)$$

The corresponding direction cosine,  $l'$ , of the angle between the line of sight and the opposite stream is

$$l' = -l = -a \cos \omega t - b \quad (2)$$

Note that

$$a + b = \cos |\theta - i|$$

$$\text{and} \quad b - a = \cos (\theta + i) \quad (3)$$

The Doppler shift is simply

$$1 + z = \gamma(1 + lv) \quad (4)$$

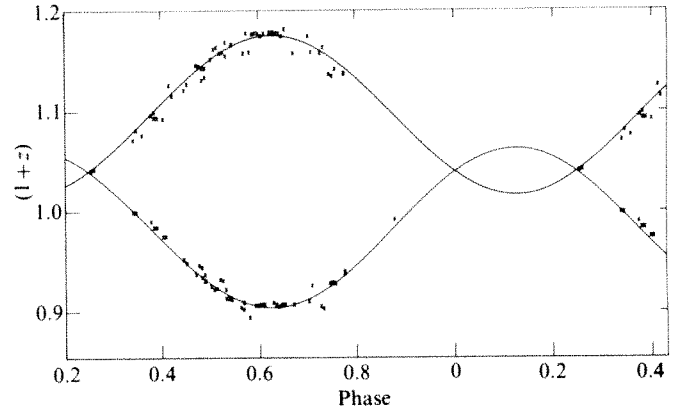
where  $l$  must be the direction cosine in the frame of the observer, and  $\gamma = (1 - v^2)^{-1/2}$ . The observed Doppler shifts of the streams with direction cosines  $l$  and  $l'$  are thus:

$$(1 + z) = \gamma(1 + va \cos \omega t + vb)$$

$$(1 + z') = \gamma(1 - va \cos \omega t - vb) \quad (5)$$

Thus  $va$  and  $vb$  are determined conveniently by observations of the ratio of  $(1 + z)$  to  $(1 + z')$  at any two phases and  $v$  follows from equations (5).

Figure 1 shows the measured Doppler shifts of SS433 obtained on 55 nights between June 1978 and April 1979, and folded with a 164-d period. These data were obtained from refs 5, 6 and 8, as well as re-interpretation of data<sup>4,12</sup> where wavelengths of the moving features were tabulated, but not previously interpreted as the Doppler effect. Note that a con-



**Fig. 1** Red- and blueshifts of SS433, measured on 55 nights during 1978–79, from sources cited in the text. The data have been folded with a 164-d period, and slightly more than one cycle is shown for clarity. The phase convention is arbitrary; as shown here, phase zero occurs on approximately 17 January 1979. The solid line is the prediction of the theoretical model described in the text, for beam velocity  $v = 0.270$ .

tiguous 35% portion of the entire cycle has never been observed. We take the point of largest amplitude of the radial velocity curves to correspond to the phase where  $\cos \omega t = 1$  (a choice of  $\cos \omega t = -1$  is simply a rotation of coordinates through  $180^\circ$ , and does not change the solution). Then the phase  $\cos \omega t = 0$  occurs at one-fourth cycle on either side of the maximum, and is a convenient second measurement point in our data. From Fig. 1 we find that the amplitudes of the radial velocity curves at the two relevant phases are: for  $\cos \omega t = 1$ ,  $(1 + z) = 1.175 \pm 0.005$  and  $(1 + z') = 0.903 \pm 0.005$ ; and for  $\cos \omega t = 0$ ,  $(1 + z) = 1.095 \pm 0.007$ ,  $(1 + z') = 0.982 \pm 0.005$ . We thus derive  $va = 0.0765$ ,  $vb = 0.0544$  and  $v = 0.270$ .

We cannot rule out a zero-point offset,  $\Delta$ , in the observed velocities due either to a cosmological (Hubble) redshift or gravitational redshift. Note, however, that the solution for  $va$  and  $vb$ , as described above, is independent of  $v$ . Because each depends on ratios  $(1 + z)/(1 + z')$ ,  $va$  and  $vb$  are also very insensitive to choices of moderate values of  $\Delta$ . Thus  $va$  and  $vb$  are well determined; a choice of  $\Delta$  determines  $v$ , and vice versa, but a range of values of  $\Delta$  and  $v$  are compatible with the data. However, a value of  $v > 0.270$  requires a positive value of  $\Delta$ , which if cosmological, requires that the system of stationary lines arises from gas with a velocity  $c\Delta$  in our direction, accidentally cancelling  $\Delta$ , and which if gravitational, poses severe difficulties in finding astrophysical mechanisms for the emission. To minimise such astrophysical problems and assumptions, we adopt  $\Delta = 0$  and  $v = 0.270$ . Then equations (5) generate the variation of  $(1 + z)$  and  $(1 + z')$  with phase, shown as smooth curves in Fig. 1.

We find the angles  $i$  and  $\theta$  from equations (3), but cannot tell which is which, that is  $(i, \theta) = (78^\circ, 17^\circ)$  or  $(17^\circ, 78^\circ)$ . The fact that the beams are found to lie at least  $61^\circ$  from the line of sight at all phases implies that any 164-d phase dependence of the moving system linewidths, caused by viewing the angular spread of the beam from different orientations, should be less than 13%, too small to be observable in our data sample.

Equations (5) also describe the situation<sup>9</sup> in which a rapidly revolving disk or ring of gas in a plane is illuminated by two pencil beams of ionising radiation from the central object, with a 164-d period of rotation. In this case, however, because the velocity variations are always confined to this plane,  $z = z'$  at  $\cos \omega t = 0$ , contrary to the observations of Fig. 1. Hence these ring or disk models must be ruled out.

In conclusion, Fig. 1 predicts that on or about 1 July 1979 the two moving emission line systems in SS433 will briefly merge.

into one, similar to a previously reported episode<sup>6</sup>, and that for the following 40 days the lines will separate again, but by an amount much less than previously observed.

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## Is Cassiopeia A a black hole?

It is rather curious that no European astronomer observed the supernova which occurred in our Galaxy in about 1668 and left the remnant Cassiopeia A. However, if the absolute magnitude of the supernova is  $M_V = -19$  mag, the interstellar absorption is  $A_V = 4.3$  mag (ref. 1) and the distance is 2.8 kpc. Then its maximum visual magnitude should have been about  $-2.5$  mag. Even  $\sim 100$  d after the event, the supernova should have been as bright as any star in the Cassiopeia constellation. The fact that no such object was recorded suggests that the optical luminosity of the supernova may have been several orders of magnitude lower than the usual value. This note argues that the low luminosity of Cas A may be an indication that the product of the explosion is a black hole.

Recently Chevalier<sup>2</sup> hypothesised that Cas A was a 'dwarf' supernova born in the explosion of a fairly massive compact star whose photosphere extended not more than  $10^{12}$ – $10^{13}$  cm and which had lost its hydrogen-rich envelope during its evolution. The fact that pre-type I-supernovae are also compact objects which lost their hydrogen envelopes during their evolution<sup>3</sup> conflicts with that hypothesis. Formation of a rapidly rotating magnetised neutron star (that is, a pulsar) during the collapse causes powerful optical emission from supernovae of this type. It is the pulsar that is pumping energy into the expanding and adiabatically cooling shell of the exploded star. Recent calculations<sup>4</sup> have shown that such a 'slow' pumping of energy (as compared with the instantaneous explosion assumed in the previous models) can ensure the light curve observed for type I supernovae.

Since no sufficiently powerful optical emission was observed in the 'compact'-supernova event of 1668, we must conclude that, for some reason, it was not accompanied by the formation of a neutron star. It follows from X-ray radiation analysis of Cas A (ref. 5) that much gas ( $3$ – $6 M_\odot$ ) was ejected during the explosion.

All these observations indicate that the star which exploded and gave birth to Cas A was a very massive object. The analysis of X-ray observations, for example, implies that the mass of the hydrogen-rich shell which the star lost in the pre-explosion stage was at least  $10 M_\odot$ . Therefore, when it had been on the main sequence its mass was not less than  $20 M_\odot$ . This accounts for the unique character of the Cas A source: although it is already

about 300 yr old, there is no source brighter than Cas A among a dozen younger sources in the Galaxy. Why it is unique is obvious: very massive stars seldom form.

The example of Cas A shows that high mass stars first detach their hydrogen-rich shells and then—after about  $10^5$  yr—they explode without giving birth to a neutron star and without the associated intense optical outburst. (Note that if a neutron star had formed after the Cas A explosion a point source of soft X-ray radiation would be observed there; a neutron star could not cool down to less than  $3 \times 10^6$  K in  $300$  yr<sup>6</sup>.)

Thus after the explosion the star must either have completely dispersed, or most of its mass must have collapsed into a black hole. It is not yet possible to choose between these alternatives; but it is likely that in Cas A the gravitational collapse ended in the formation of a black hole. First, it follows from the relatively small mass of the hydrogen-depleted part of the exploded star (the part observed as fast moving filaments) as compared with the earlier-detached hydrogen rich shell. The chemical composition of the material in the fast-moving filaments is the most important argument favouring the conclusion that a black hole formed during the Cas A supernova event. The absence of elements of the Fe-group is highly characteristic of the spectrum observed for the fast-moving filaments. However, theoretical calculations of the star's expansion<sup>7</sup> lead us to expect that at least  $\sim 1 M_\odot$  of the exploded star should have transformed into Fe-group elements. In that case the spectrum of fast-moving filaments would inevitably have Fe II lines, in particular, the intense  $\lambda 4570$  line.

Thus the observations strongly support the conclusion that a black hole formed after the Cas A outburst.

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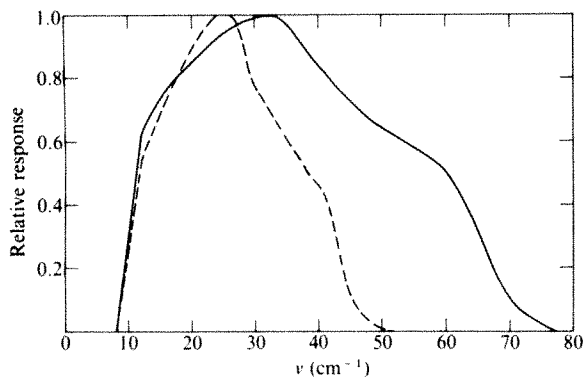
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## Millimetre and submillimetre measurements of the Crab Nebula

THE flux from the central region of the Crab Nebula in three broad spectral passbands with flux-weighted mean wavelengths of 300  $\mu$ m, 400  $\mu$ m and 1 mm have been measured. We report here that the inferred total flux densities for the entire nebula (Table 1) are consistent with an extrapolation of the power law spectrum found at longer wavelengths<sup>1</sup>.

The 300 and 400  $\mu$ m observations of the Crab Nebula were made with the University of Chicago f/13 photometer on the NASA Kuiper Airborne Observatory in September 1978. The detector was a Ga-Ge bolometer with heat trap field optics<sup>2</sup>. The system had a focal plane aperture of 1.9' and a chopper throw of 5.0'. The photometric pass bands were defined at short wavelengths by interference filters and at long wavelengths by diffraction and by the transmission characteristics of the light collector. We have measured the relative response of the photometer in the laboratory using both a grating spectrometer and a Fourier transform spectrometer. The two pass bands are shown in Fig. 1. W51 was used as the calibration source, assuming a spectrum of the form  $\nu B_\nu(T)$  where  $\pi B_\nu(T)$  is the





**Fig. 1** Relative responses of the passbands used for airborne observations. The solid and dashed curves have mean wavelengths of 300  $\mu\text{m}$  and 400  $\mu\text{m}$ , respectively.

Planck function. For a 1.9' aperture centred on the submillimetre peak of W51, we have assumed  $T = 40$  K and have normalised the function such that the flux density at 200  $\mu\text{m}$  is 24,000 Jy. These parameters give a good fit to measurements in the range  $80 \leq \lambda \leq 540$   $\mu\text{m}$  (refs 3–5) allowing for the effects of beam size. The signal-to-noise ratios for the Crab Nebula observations were 9 for the 300  $\mu\text{m}$  pass band and 3.6 for the 400  $\mu\text{m}$  pass band.

The 1-mm measurement was made with the Chicago  $f/7.5$  photometer at University of Hawaii 2.2 m telescope at Mauna Kea in September 1977. This detector also uses a Ga-Ge bolometer with heat trap field optics. The focal plane aperture and chopper throw were 3.2' and 8.0' respectively. The spectral response was defined by a helium temperature filter of fluorogold and black polyethylene, by the transmission of the atmosphere, and by diffraction. Jupiter was used as the calibration object with an assumed 750  $\mu\text{m}$  brightness temperature of 162 K (ref. 6). The Crab Nebula observations had a signal-to-noise ratio of  $> 6$ .

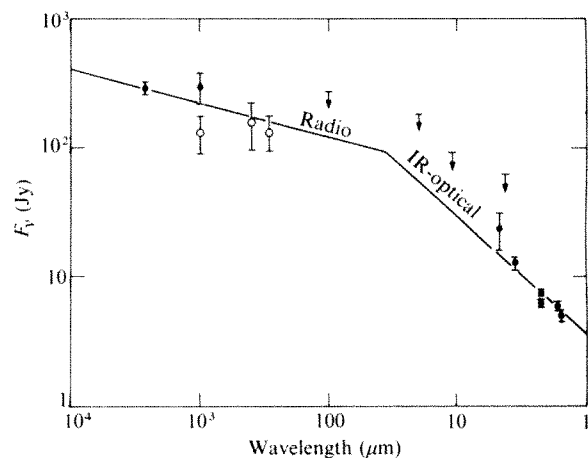
We derived flux densities from the observed signals by the methods described in ref. 7. The spectrum of the Crab Nebula was assumed to be proportional to  $\nu^{-0.26}$  in agreement with the radio spectrum; a change of  $\pm 0.25$  in the assumed spectral index produces a change of  $< 3\%$  in the derived flux densities. To allow for the effects of diffraction on the spectral response, we have assumed a gaussian source profile, convolved the assumed source with a diffraction pattern, and calculated the fraction of radiation within the focal plane aperture as a function of wavelength. The total flux densities in Table 1 were derived assuming a source size of  $2.3 \times 3.6'$  FWHM<sup>8</sup>. Errors of  $\pm 0.3'$  in both source dimensions result in errors of  $\leq 10\%$  in the obser-

ved flux density, but lead to errors of 15–20% in the extrapolation to total flux density. The estimated error in absolute calibration is  $\approx 20\%$ .

Werner *et al.*<sup>9</sup> have also measured the total flux density of the Crab Nebula at 1 mm. Their value,  $300 \pm 80$  Jy, is much larger than ours. The discrepancy is due primarily to the different methods used to obtain the total flux density. Werner *et al.* used a 1' beam to map the source and derived a total flux density from integration of their map. Their map shows a source with FWHM of  $4.5' \times 2.5'$ , although this size is somewhat uncertain due to the low signal-to-noise ratio in the outer regions of the map. The larger size leads to a higher total flux density. If we derive instead a total flux density from their observed central surface brightness and our assumed source size, we get a value of 210 Jy, in much better agreement with the value in Table 1. The question of the source size at wavelengths  $\leq 1$  mm deserves further study.

The total flux densities in Table 1 are consistent with a continuation of the radio spectrum into the submillimetre region with no change in slope (Fig. 2) although the errors would allow a change in slope of 0.25. However, the present data can be used to exclude more radical departures from the extrapolated radio spectrum. Kirshner<sup>10</sup>, for example, has suggested that the radio spectrum might steepen above 15 GHz. The resulting submillimetre flux densities would lie almost a factor of 3 below our measured values.

The present data show no increase in flux density at short-wavelengths which could be attributed to thermal radiation from



**Fig. 2** Spectrum of the Crab Nebula. Data sources: ○, this paper; 2.7 mm, ref. 8; 1 mm, ref. 9; 20  $\mu\text{m}$ , 11  $\mu\text{m}$  and 4.2  $\mu\text{m}$ , ref. 12; 4.75  $\mu\text{m}$ , 2.2  $\mu\text{m}$ , 1.65  $\mu\text{m}$  and 1.55  $\mu\text{m}$ , ref. 13; 3.5  $\mu\text{m}$  and 2.2  $\mu\text{m}$ , ref. 14; 100  $\mu\text{m}$ , ref. 15; radio and IR-optical fits, ref. 1.

**Table 1** Submillimetre flux density of the Crab Nebula

Mean wavelength ( $\mu\text{m}$ )	Observed flux density (Jy)	Total flux density (Jy)
300	$35 \pm 8^*$	$135 \pm 41^\dagger$
400	$41 \pm 14^*$	$158 \pm 63^\dagger$
1,000	$75 \pm 19^\ddagger$	$131 \pm 42^\dagger$

\* Into a 1.9' aperture. The error includes a 20% contribution from uncertainties in absolute calibration and in the assumed source spectrum.

† Assuming a gaussian source with FWHM of  $2.3' \times 3.6'$ . The error includes an additional 20% contribution due to the extrapolation to total flux density.

‡ Into a 3.2' aperture. The error includes a 20% contribution from uncertainties in absolute calibration, assumed source spectrum and atmospheric water vapour.

dust. From our measurement at 300  $\mu\text{m}$  we may place an upper limit on the dust emission within the range  $170 < \lambda < 900$   $\mu\text{m}$  equal to the observed flux,  $\nu F_\nu < 1.3 \times 10^{-12}$   $\text{W m}^{-2}$ . For dust spectra which have their peaks in this wavelength range, corresponding to  $T \leq 20$  K, our result improves the UCL limit of  $2 \times 10^{-10}$   $\text{W m}^{-2}$  between 40 and 350  $\mu\text{m}$ <sup>11</sup>.

At 100  $\mu\text{m}$  the best limit on the flux of the Crab Nebula is  $F_\nu < 275$  Jy<sup>15</sup>, which gives  $\nu F_\nu < 10^{-11}$   $\text{W m}^{-2}$ . We conclude that the luminosity of dust emission from the Crab Nebula is less than  $1300 L_\odot$  for dust with temperatures  $\leq 40$  K, but that there could be as much as  $5 \times 10^3 L_\odot$  emitted by dust with  $T \sim 120$  K.

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## Acoustic absorption by $\text{MgCO}_3^0$ ion-pair relaxation

UNDERWATER sound propagation measurements in natural bodies of water frequently reveal attenuation anomalies that cannot be ascribed to known processes. When chemical relaxations are suspected, the acoustic resonator-decay method has proved an effective means of investigation in laboratory conditions. By stepwise synthesis of an artificial medium of similar composition and measuring changes in decay rates, the responsible chemical constituents can be identified. Then by combined stoichiometry and acoustic measurements with different concentrations, the relaxation kinetics of the reacting species can be determined. We have shown previously by resonator measurements that the acoustic absorption in Lake Tanganyika is the result of a chemical relaxation involving magnesium and carbonic acid<sup>1</sup>. The same relaxation is also believed to be responsible for one of the absorption components in seawater<sup>2</sup>. We show here that the relaxation kinetics follow a two-step association of the magnesium carbonate ion-pair.

If activity effects are neglected, the absorption arising from the slower second step of the two-step equilibrium  $\text{A} + \text{B} \rightleftharpoons \text{C} \rightleftharpoons \text{D}$  can be written

$$\alpha = \alpha_{\max} f^2 / (f^2 + f_r^2) \quad (1)$$

where  $f$  is the acoustic frequency and

$$\alpha_{\max} = \frac{(\Delta V)^2 k_2^- K_2 (C + D)}{2\beta_0 RT (1 + K_2)} \text{ neper s}^{-1} \quad (2)$$

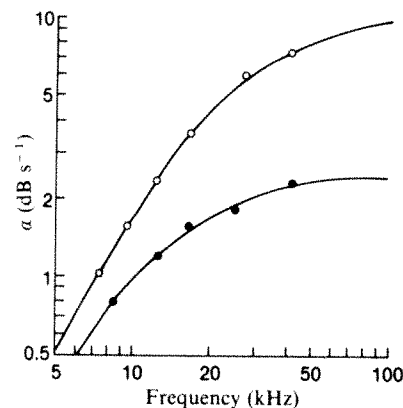


Fig. 1 Measured decay rates against frequency for  $[\text{NaCl}] = 0.4 \text{ M}$  and  $[\text{CO}_2] = 2.5 \text{ mM}$ . ●,  $[\text{Mg}] = 5 \text{ mM}$ ,  $\text{pH} = 9.0$ ,  $pK'_{2C} = 9.56$ . ○,  $[\text{Mg}] = 50 \text{ mM}$ ,  $\text{pH} = 8.7$ ,  $pK'_{2C} = 9.17$ .

The respective concentrations are given by  $A$ ,  $B$ ,  $C$  and  $D$  and the equilibrium constants by  $K_1 = C/AB$  and  $K_2 = D/C$ . The relaxation frequency is given by

$$f_r = \frac{k_2^-}{2\pi} \left[ \frac{1 + K_1(1 + K_2)(A + B)}{1 + K_1(A + B)} \right] \text{ Hz} \quad (3)$$

where  $k_2^-$  is the reverse rate constant of step two and  $K_2 = k_2^+ / k_2^-$  where  $k_2^+$  is the forward rate constant. The effective change in molar volume is given by  $\Delta V = \Delta V_2 + \Delta V_1 / (1 + K_1(A + B))$  where  $\Delta V_1$  and  $\Delta V_2$  are the molar volume changes of the respective steps. Other constants are: compressibility,  $\beta_0 = 1/\rho_0 c_0^2$  where  $\rho_0$  is density and  $c_0$  is the speed of sound; gas constant,  $R$  and absolute temperature,  $T$ .

In the present case let  $A = [\text{Mg}^{2+}]$ ,  $B = [\text{CO}_3^{2-}]$  and  $C + D = [\text{MgCO}_3^0]$ . As  $\text{pH} > 7$  is the domain of interest, the carbonate-bicarbonate equilibrium is the only concern<sup>4</sup>. The equilibrium constant is given by  $K_{2C} = a_H[\text{CO}_3^{2-}] / [\text{HCO}_3^-]$  where  $a_H$  is the hydrogen ion activity.

To avoid inherent problems at low ionic strengths, all measurements were made in 0.4 M NaCl solutions. If other minor ion-pairings are neglected, the apparent equilibrium constant can be written<sup>5</sup>

$$K'_{2C} \approx K_{2C} (1 + K_1^* [\text{Mg}^{2+}] + K_2^* [\text{Na}^+]) \quad (4)$$

where  $K_1^* = [\text{MgCO}_3^0] / [\text{Mg}^{2+}][\text{CO}_3^{2-}]$  and where  $K_2^* = [\text{NaCO}_3^-] / [\text{Na}^+][\text{CO}_3^{2-}]$ . From this we have

$$[\text{MgCO}_3^0] \approx [\text{CO}_2] \frac{x}{1+x} \frac{y}{1+y_0+y} \quad (5)$$

where  $x = K'_{2C} / a_H$ ,  $y = K_1^* [\text{Mg}^{2+}]$  and  $[\text{CO}_2]$  is the total concentration. The apparent equilibrium constants were measured in a solution containing equal concentrations (1 mM) of  $\text{NaHCO}_3$  and  $\text{Na}_2\text{CO}_3$  which gives  $K'_{2C} \approx a_H$  or  $pK'_{2C} \approx \text{pH}$  ( $\text{pH}$  was measured by means of an  $\text{Ag}/\text{AgCl}$  electrode). The value  $y_0 = K_2^* [\text{Na}^+]$  was determined from the change in  $K'_{2C}$  when the NaCl was added. The value of  $K_1^*$  was then determined from the slope of  $K'_{2C}$  against  $[\text{Mg}^{2+}]$  measured when  $\text{MgCl}_2$  was added in steps. In the range of concentrations being considered, we have approximated  $[\text{Mg}^{2+}] \approx [\text{Mg}]$  where  $[\text{Mg}]$  is the total concentration<sup>6</sup>.

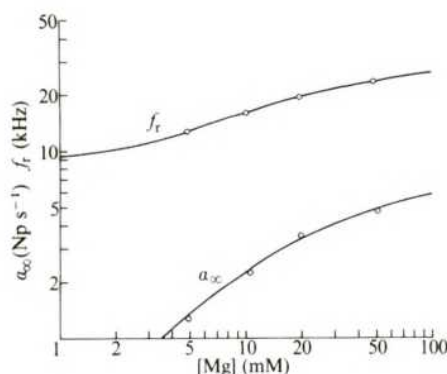
Our attention has been drawn to ref. 6 which shows that  $\text{MgCl}^+$  ion-pairing may also be important. To account for the reduced free ion concentration we can write  $[\text{Mg}^{2+}] \approx \text{constant} \times [\text{Mg}]$ . However, as  $K_1^*$  was measured in identical conditions, its value must be correspondingly increased which leaves the results of equation (5) unchanged.

All measurements were made at 22 °C. The resonator apparatus and procedures are described in ref. 2.



Figure 1 shows typical measurements of decay rates against frequency. The data are fitted by equation (1) which gives  $\alpha_{\max}$  and  $f_r$  for each set of experimental conditions. The  $\alpha_{\max}$  values were normalised by the formula  $\alpha_{\infty} = \alpha_{\max}(1+x)/x$ . Thus  $\alpha_{\infty}$  is the value one would have for  $pH \gg pK'_{2C}$ , that is, if all  $CO_2$  were in the form of the three carbonate species. The values were converted from  $dB\ s^{-1}$  to  $Np\ s^{-1}$  by dividing by 8.7.

The  $\alpha_{\infty}$  and  $f_r$  data are shown in Fig. 2. The  $f_r$  data were fitted by equation (3) using the measured value  $K_1^* = K_1(1+K_2) = 160/M$  with  $K_2 = 2$  and  $k_2^- = 6 \times 10^4\ s^{-1}$ . The  $\alpha_{\infty}$  data were fitted



**Fig. 2** Absorption  $\alpha_{\infty}$  and relaxation frequency  $f_r$  plotted against magnesium concentration for 0.4 M NaCl solution. Similar curves would be obtained for Lake Tanganyika conditions except that the [Mg] scale would be roughly an order-of-magnitude smaller because of the much greater values of  $K_1^*$  at low ionic strengths.

using the value  $\Delta V = \Delta V_2 = 12\ ml/M$  in equation (2) and the measured value  $y_0 = 3.5$  with  $x \gg 1$  in equation (5).

The agreement between theory and experiment shows that the absorption mechanism in question can be modeled as the two-step  $MgCO_3$  equilibrium in which the slower second step controls the relaxation process. The calculated rate constants should be valid for both Lake Tanganyika and seawater at 22 °C. It appears, therefore, that the earlier estimate of the Lake Tanganyika relaxation frequency (40 kHz) is too high. Present measurements indicate that it should be no greater than 30 kHz.

Calcium ion-pairing may not entirely account for the reduced absorption when  $CaCl_2$  is added<sup>1,2</sup>; hence, a decrease in  $\Delta V_2$  may also be involved. In seawater, calcium evidently provides the coupling between the magnesium carbonate and the boric acid/borate equilibria that is responsible for an order of magnitude increase in the relaxational absorption of the latter<sup>7</sup>.

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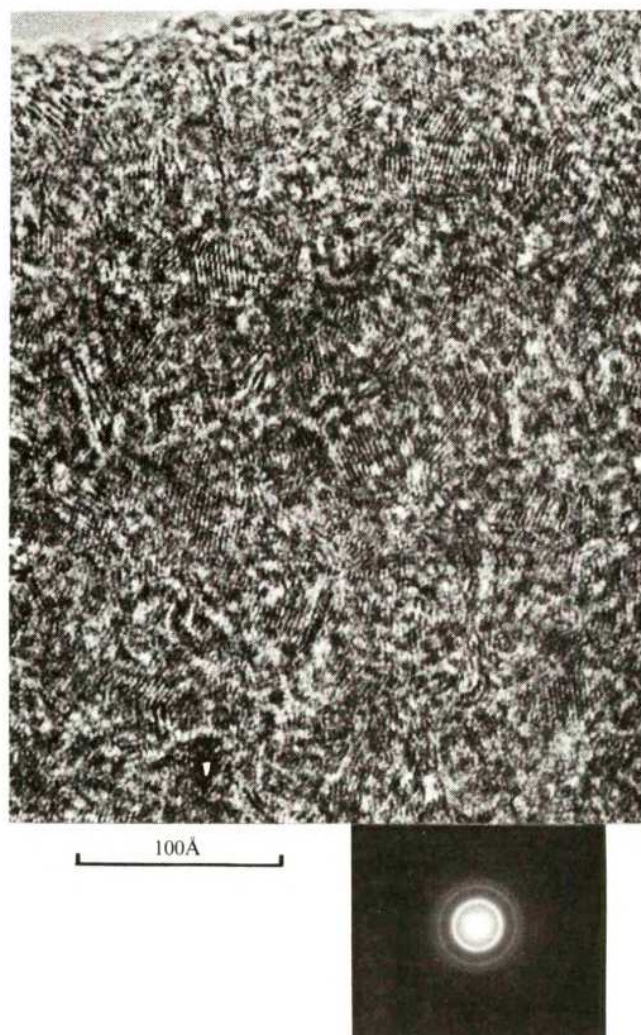
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## Anomalous characteristics of the microcrystalline state of SiC fibres

FIBRES of SiC are prepared<sup>1-4</sup> from the polycarbosilane which, in various forms, is made into a precursor by melt-spinning: the precursor is then heated to produce the SiC fibre. Ceramics are produced in this way by heating the organosilicon polymer. The organic precursor is prepared in a subtle form (for example, as fibres) which is extremely difficult to produce from an inorganic precursor. On heating the organic precursor a ceramic skeleton remains. This inorganic residue derived from an organosilicon polymer possesses characteristics not previously known and these are reported here.

Table 1 shows a chemical analysis of the SiC fibre obtained after heat-treatment (1,300 °C) of the precursor fibre. Its atomic ratio is Si:C:O:H = 1:1.46:0.36:0.03. Table 1 shows that the SiC fibre contains appreciable amounts of carbon and of oxygen which had been introduced by the heating of the precursor fibre. Assuming then that the oxygen is present in the fibre as  $SiO_2$ , the molar ratio of compounds in the SiC fibre is  $SiC:C:SiO_2 = 1:0.78:0.22$ .

The apparent density of the  $\beta$ -SiC fibre obtained by heating a precursor fibre at 1,300 °C is  $2.6\ g\ cm^{-3}$ , which is lower than the true density  $3.2\ g\ cm^{-3}$  of  $\beta$ -SiC.



**Fig. 1** High resolution electron micrograph of a precursor fibre heat-treated at 1,300 °C.



**Table 1** Composition (wt%) of fibres

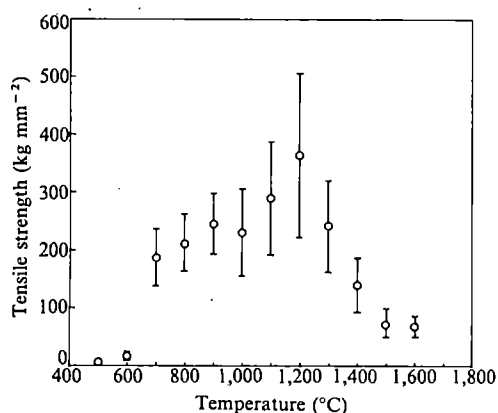
	Si	C	O	H
Precursor fibre	46.8	38.1	0.82	7.40
Fibre after curing	44.4	31.0	15.5	5.30
SiC fibre	50.5	31.6	10.3	0.055
SiC fibre after heat treatment at 1,250 °C	51.3	23.9	18.5	0.015

The SiC fibre possesses a high tensile strength ( $350 \text{ kg mm}^{-2}$ ), a diameter of  $10 \mu\text{m}$  and a high Young's modulus ( $20 \text{ tonnes mm}^{-2}$ ). The fibre sustains a load of  $200 \text{ kg mm}^{-2}$  at  $1,250^\circ\text{C}$  for 3 d in air. Table 1 shows a chemical analysis of the SiC fibre after heat-treatment. Its compound molar ratio is  $\text{SiC}:\text{C}:\text{SiO}_2 = 1:0.59:0.46$ ; a lot of carbon still remains.

The tensile strength and the Young's modulus of the SiC fibre are independent of temperature (between room temperature and  $1,400^\circ\text{C}$ ) *in vacuo*<sup>5</sup>, and the strength at the high temperature in air<sup>6</sup> is as good as other silicon carbide fibres<sup>7,8</sup>.

$\text{SiO}_2$  is considered mainly to exist at the surfaces of the SiC fibre. We investigated how the SiC and the excess carbon come to exist in the fibre.

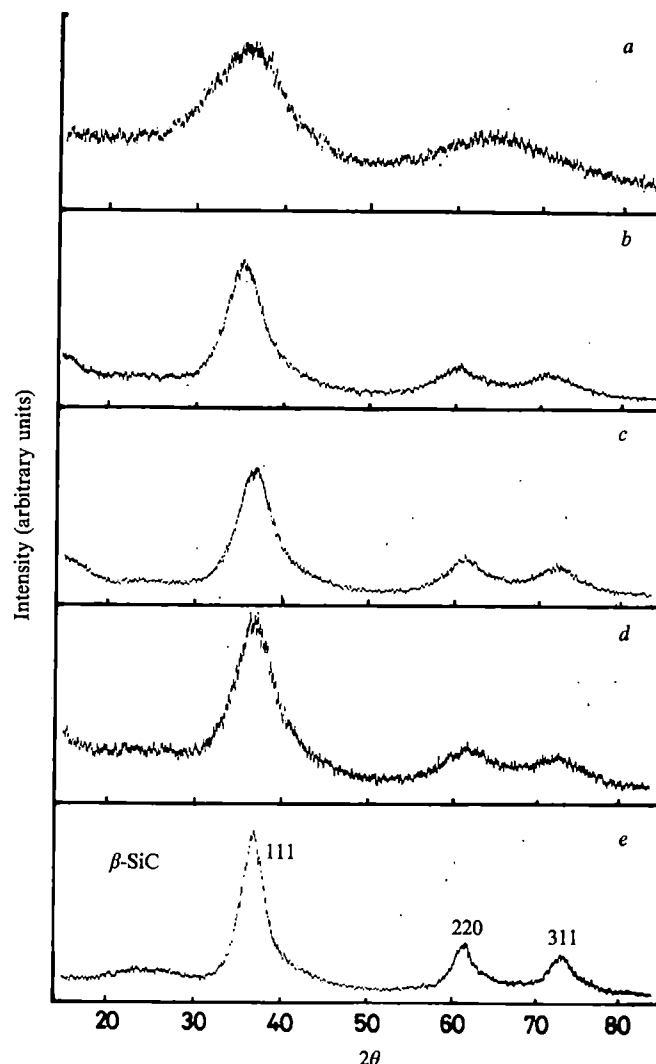
It has been previously reported that in the SiC fibre, the SiC matrix is distributed uniformly as fine grains from the result of X-ray diffraction<sup>1-4</sup>. Figure 1 shows a high-resolution electron



**Fig. 2** Variation of the tensile strength of precursor fibres with heat-treatment temperatures.

micrograph of the powdered SiC fibre. The fibre was prepared by heating a precursor fibre at  $1,300^\circ\text{C}$  in a vacuum. The lattice image corresponding to a  $2.5 \text{ \AA}$  interlayer spacing of  $\beta\text{-SiC}$  (111) planes and a  $3.4 \text{ \AA}$  interlayer spacing of graphite (002) planes are observed. The SiC is ultrafinely grained. Figure 1 also indicates a uniform distribution of fine grains of carbon surrounded by  $\beta\text{-SiC}$ . It is difficult for the fibre in this condition to be oxidised. A high-resolution electron micrograph of SiC fibre after the  $1,250^\circ\text{C}$  heat-treatment was also taken. The distribution of carbon was almost the same as before the heat treatment.

The excess carbon and the oxygen introduced in the curing process may affect the microcrystalline state of  $\beta\text{-SiC}$ . The relationship between the mechanical strength of the fibre and the heating temperatures for the precursor fibre *in vacuo* is shown in Fig. 2. Heating at  $1,500^\circ\text{C}$  causes the mechanical strength to drop, as shown in Fig. 2. Figure 3 shows the X-ray (Ni-filtered  $\text{Cu K}\alpha$ ) diffraction patterns of the precursor fibre heated at  $1,000$ ,  $1,100$ ,  $1,200$ ,  $1,300$  and  $1,500^\circ\text{C}$ . The X-ray diffraction patterns on heating at  $1,500^\circ\text{C}$  indicate a larger fraction of crystallised  $\beta\text{-SiC}$  in comparison with the patterns of fibres heated at  $1,100$ – $1,300^\circ\text{C}$ . The mechanical strength drops with crystallisation of the  $\beta\text{-SiC}$ . The stable and uniformly distributed carbon in the SiC fibre may be precipitated with



**Fig. 3** X-ray diffraction patterns of precursor fibres heat-treated at various temperatures. Full-scale intensity of patterns at  $1,100$  (b);  $1,200$  (c); and  $1,500^\circ\text{C}$  (e) is twice that of patterns at  $1,000$  (a) and  $1,300^\circ\text{C}$  (d).

crystallisation of the  $\beta\text{-SiC}$  phase, hence lowering the mechanical strength: in other words, the SiC fibre in the microcrystalline state has high mechanical strength.

Chemical analysis of the SiC fibre and its high-resolution electron micrograph show an unexpectedly large quantity of excess carbon existing in the fibre. In spite of this, this fibre has a high strength and a high heat-resistance at high temperatures.

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## Spontaneous formation of lecithin bilayers at the air–water surface

THE energetics of formation of various surface film states of dimyristoyl lecithin (DML) has been recently obtained from equilibrium spreading pressure  $\pi_e$  measurements<sup>1</sup>. One result from that study, but not reported then, was the formation of a new equilibrium lecithin surface phase. Its existence was deduced solely from formal thermodynamic arguments based on the Gibbs phase rule, and therefore reporting of the new surface phase was deferred until more direct experimental evidence was available for describing its properties. We have now characterised this new surface phase by measuring the surface concentration of DML films and have found the concentration to be double that of a close-packed monomolecular film. Before presenting these results and their implications, we summarise the arguments which led to the prediction of the new lecithin surface phase.

In principle,  $\pi_e$  measurements as a function of temperature indicate the existence of all phase transitions; they also provide a thermodynamic basis for calculating transition energies<sup>2–4</sup>. The equilibrium surface phase relations for DML obtained by this approach in the region of  $T_c$ , the gel–liquid crystal transition temperature, is given in Fig. 1a. Line ABCD represents the coexistence line for the equilibrium of surface film with bulk DML, either gel or liquid crystal. Point B is at 23.5 °C,  $T_c$  for DML. At temperatures below  $T_c$ , the bulk gel state is in equilibrium with a gaseous surface film; at temperatures above  $T_c$  liquid crystal is in equilibrium with a gaseous film which changes with increasing temperatures into a liquid expanded film along line CD. Line CE represents the equilibrium of liquid expanded film with surface gas in the absence of bulk lipid; these values of  $\pi$  are the equivalent of the surface vapour pressure<sup>1</sup>. Line CE' represents the supercooled liquid expanded film surface vapour pressure; it is formed when solvents are used to form the film at temperatures below 25 °C (ref. 1).

The phase line CD which describes the equilibrium between liquid crystal and liquid expanded film indicates that  $\pi_e$  increases rapidly with temperature. But  $\pi_e$  must reach a finite limit at some elevated temperature. Indeed, measurements of  $\pi_e$  for DML at temperatures exceeding 25 °C, shown in Fig. 1, indicate that  $\pi_e$  reaches a maximum of 49.5 dyn cm<sup>–1</sup> at about 29 °C. With further increase in temperature  $\pi_e$  decreases slightly. Similar results were obtained by Phillips and Hauser<sup>5</sup>.

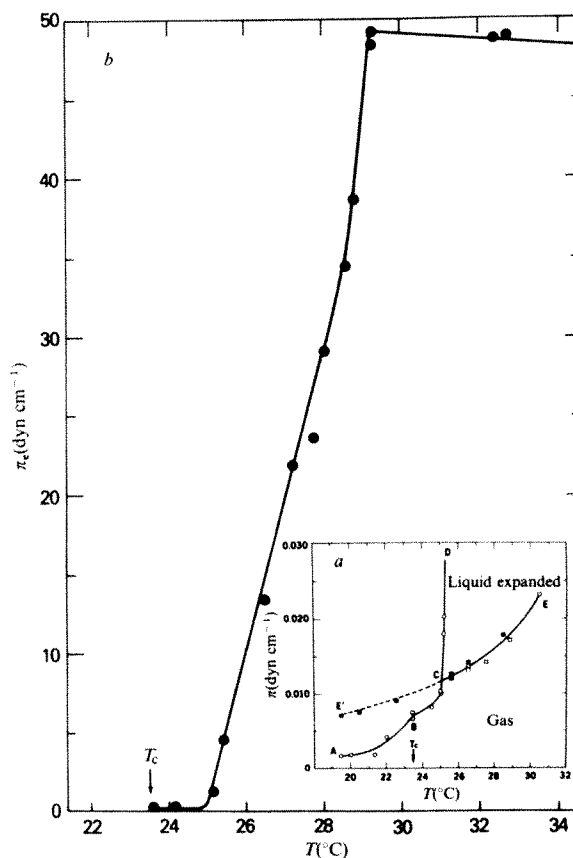
The Gibbs phase rule at constant atmospheric pressure for this system<sup>6</sup> may be written

$$F = C - P_s - P_b + 2$$

where  $C$  is the number of components (water, lecithin, and air),  $P_s$  and  $P_b$  are the number of surface phases at the air–water surface, and bulk phases, respectively, and  $F$  is the number of intensive variables (temperature and chemical potential) which may be varied independently. For this system, with bulk lipid and at least one surface phase always present,  $F$  is either 1 or 0. Thus, when  $\pi_e$  varies with temperature,  $F = 1$  and the chemical activity of lipid in each equilibrium phase also varies with temperature. Examples are given in Fig. 1a, along line AB where  $P_b = 3$  (gel, water, air),  $P_s = 1$  (surface gas), and along line BC where the bulk lipid gel state becomes liquid crystal at the elevated temperatures. At the gel–liquid crystal transition temperature, point B (Fig. 1),  $F = 0$ , as  $P_b = 4$  (gel, liquid crystal, water, air), and  $P_s = 1$ .

At temperatures above and below 29 °C (the temperature where  $\pi_e$  is a maximum)  $\pi_e$  is also temperature dependent,  $F = 1$ , and therefore homogeneous surface films exist both below and above 29 °C. However, at 29 °C  $F = 0$ , and the phase rule therefore indicates that a phase transition occurs just as at

point B (Fig. 1); this new phase transition may be either in the surface or bulk. Because the temperature where this occurs exceeds  $T_c$ , the only lipid bulk phase present is the liquid crystal. As there is no evidence for a DML bulk phase transition at 29 °C, we deduce that at the temperature where  $\pi_e$  is a maximum, DML forms a new surface phase. To establish that the new phase which appears at 29 °C is in the surface, DML surface concentrations were measured by two independent methods.



**Fig. 1** a, Surface pressure  $\pi$  as a function of temperature in the region of the gel–liquid crystal transition temperature,  $T_c$  for DML. For explanation of phase diagram see text, and ref. 1. b, Complete equilibrium spreading pressure  $\pi_e$ –temperature diagram for DML. Note change in scale for  $\pi$ . Wilhelmy plate method used for this data, sensitivity of the order of 0.1 dyn cm<sup>–1</sup>. Data for the figure inset were obtained by the millidyne balance<sup>1</sup>. Therefore the details seen in the inset are not observed in the expanded range figure.

The first method entails isolating and collecting the surface film which accumulates in the air–water surface when DML crystals are placed on the water surface. A temperature controlled ( $\pm 1^\circ$ ) film balance trough was divided into two compartments by a strip of Teflon. The strip has a slit on its upper edge to allow a small connection at the surface between the two water compartments. Bulk lipid was placed on the water surface of one compartment and held in place by a platinum wire loop; the second compartment was monitored for changes in  $\pi$  by the Wilhelmy plate technique. The bulk lipid generally remained effectively trapped behind the Teflon strip separating the two compartments. Films which accumulate at the surface of the second compartment were swept by a Teflon barrier over the edge of the trough and across a collection spout into beakers. The water which is entrained with the lipid amounts to about

0.01 ml cm<sup>-2</sup> of surface film<sup>7</sup>. This water was evaporated and the dried phospholipid was analysed for phosphorus<sup>8</sup>. Generally 350–400 cm<sup>2</sup> of surface was sufficient to provide enough lipid for the analysis. The films were collected 2 and 4 h after the surface pressure had ceased to change; the time interval had no significant influence on the amount of material collected.

For the second method the surface concentration was obtained by measuring the surface radioactivity of <sup>3</sup>H-DML adsorbed from equilibrium dispersions of DML. The dispersions of DML gave identical values of  $\pi_e$  as those obtained by spreading from the crystal. A thermostatted ( $\pm 0.1^\circ$ ) glass cell was used for both the  $\pi_e$  and radiotracer measurements of the dispersions. Specific activity of <sup>3</sup>H-DML was approximately 35 Ci mol<sup>-1</sup>, (a purified sample provided by Dr R. E. Pagano). Surface radioactivity was monitored by an end-window gas-flow detector; the window was composed of Parylene<sup>9</sup> with a vacuum deposit of gold (approximate thickness: 70  $\mu$ g cm<sup>-1</sup> Parylene, 30  $\mu$ g cm<sup>-1</sup> gold). The detector was constructed by the Aloka Company, Mitaka, Japan, and was used with a standard scalar (Nuclear-Chicago, model 168). The counting efficiency of the detector was about 2% at a height of 1 mm above the water surface. Surface radioactivities were not affected by bulk particles of DML in the dispersion because the lipid density is greater than for water<sup>10</sup>, thus these particles settled out of the range of tritium ( $\sim 1 \mu$ m in water) within 30 min. More complete details of the method will be presented elsewhere. Dispersion concentrations of 0.1–1.0 mg ml<sup>-1</sup> were used and the reported surface radioactivities were found to be independent of the dispersion concentrations.

The surface concentrations of DML as a function of temperature obtained by both methods is shown in Fig. 2. The values of  $\Gamma$  obtained by the two methods are in general agreement, with the larger error of the sweeping experiments reflecting the multiple operations required for the analysis. Below 25 °C where the equilibrium surface pressure is very low (surface gas region, Fig. 1) the surface concentration is close to zero. With increasing

temperature, the surface concentration increases to a maximum value of  $6.4 \times 10^{-10}$  mol cm<sup>-2</sup> at about 29 °C. At still higher temperatures  $\Gamma$  decreases monotonically to  $3.2 \times 10^{-10}$  mol cm<sup>-2</sup> at about 35 °C.

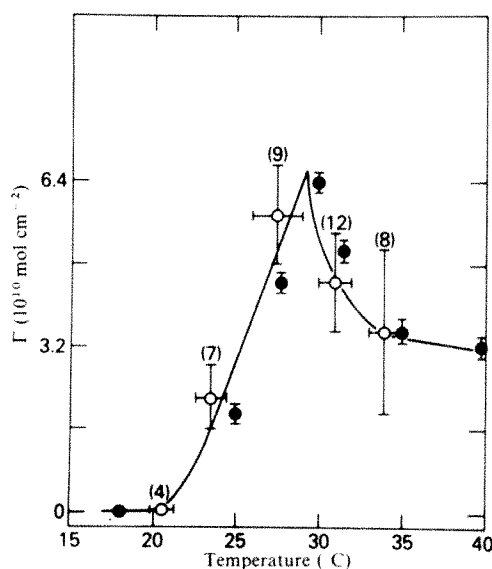
Film balance studies indicate that close-packed monolayers occupy 50–55 Å<sup>2</sup> per molecule or  $3.3\text{--}3.0 \times 10^{-10}$  mol cm<sup>-2</sup> (ref. 11). Thus the adsorbed film formed at 35 °C (Fig. 2) appears to be a close-packed monolayer; at 29 °C the surface concentration is exactly double that of the close-packed monolayer, or the equivalent of a bilayer. The formation of the surface bilayer is not fortuitously dependent on the experimental conditions because its formation is independent of the dispersion concentration, and the same results were obtained by two independent methods. Moreover, the temperature of surface bilayer formation coincides with the temperature in which  $\pi_e$  is a maximum (Fig. 1b), and for which the phase rule predicts that a new lecithin phase appears. The new surface phase predicted by the phase rule is the surface bilayer.

While we cannot ascribe molecular configurations to the surface films which are reported in these studies, hypotheses consistent with the phase rule are suggested for describing the surface phase transitions. Figures 1 and 2 indicate that DML surface films between 25 and 29 °C pass continuously from the gaseous to the bimolecular state. As the phase rule predicts that a single homogeneous surface phase is present, we assume that at each temperature in this interval states of aggregation intermediate between gaseous and bilayer are formed. Between 29 and 35 °C the data suggests that the surface bilayer reverts to a condensed monolayer structure. In this interval the phase rule indicates that the surface film is homogeneous, but that surface bilayer must also be present. We therefore deduce that bilayer is transformed continuously to condensed monolayer, but assume that monolayer which forms is completely miscible with the bilayer, that is both monolayer and bilayer coexist in a homogeneous surface phase. At temperatures exceeding 35 °C condensed monolayer is the only surface state present. Phenomenologically, DML forms in sequence with increasing temperatures: gaseous film, surface bilayer and finally condensed monolayer.

Several additional aspects of this system are noteworthy. The surface bilayer will form only in the presence of bulk phase liquid crystal, and at a unique temperature which is presumably a characteristic of each lecithin. In equilibrium conditions the surface bilayer will not exist independently of multilamellar liquid crystal. Questions on the generality of these phenomena and the thermodynamics of formation of surface bilayers will be examined in detail elsewhere. Finally, it has always been a basic tenet of lipid film studies on water that the films may be assumed to be monomolecular. We have shown that for at least one equilibrium system, surface films can exceed monolayer concentrations.

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**Fig. 2** Surface concentration of DML films as a function of temperature. ○, Film concentration obtained by sweeping method, films formed by spreading from bulk crystals. Each data point is given with standard errors of the mean, and the number of samples are shown in parentheses above the error bar. The large s.e.m. reflect the multiple operations required for the analysis and the poor temperature control ( $\pm 1^\circ$ ) inherent in the large film balance trough used. ●, Film concentration obtained by radiotracers, films formed by adsorption from dispersions containing <sup>3</sup>H-DML. Duplicate analyses gave a reproducibility of  $\pm 5\%$ , temperature controlled to  $\pm 0.1^\circ$ .

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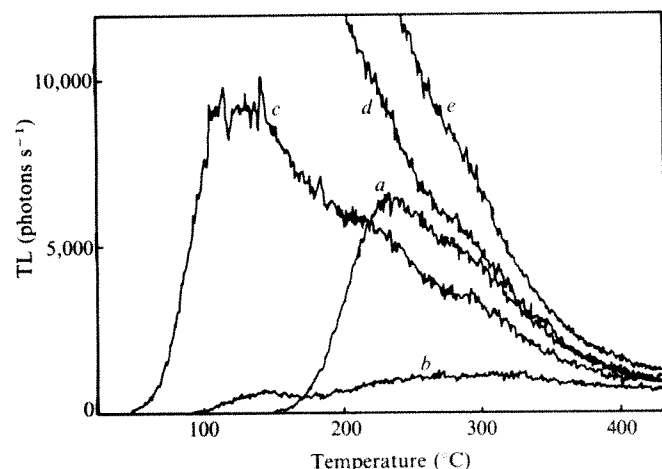
## Thermoluminescence dating of a deep-sea sediment core

IN the last decade thermoluminescence dating has been developed for use on archaeological material, principally pottery, that was heated in antiquity<sup>1</sup>. Thermoluminescence (TL) is the light emitted by a material when heated and which results from a previous dose of radiation. In the simplest cases the light intensity is proportional to the radiation dose and can be used for determining an unknown dose; when combined with other measurements which yield the dose rate the TL can thus be used to calculate an age. In the case of pottery the event being dated is the last heating of the material to a high temperature, typically 500 °C. The use of TL to date the deposition of ocean sediments which we propose here is similar in the main principle except for the lack of the heating event. We describe here the experimental evidence which indicates that some event which has the same result does occur. We then show that exposure to sunlight could be this event, and finally show that TL dates obtained for an ocean sediment core are in agreement with dates determined independently.

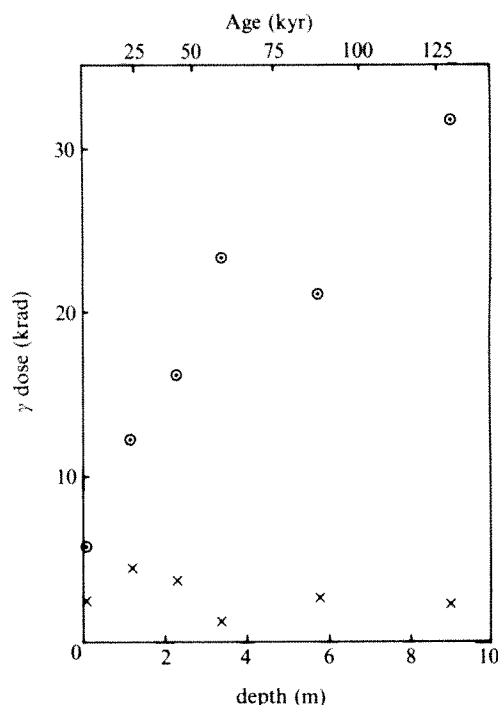
The results presented here are for 4–11- $\mu$ m grains of samples of the deep-sea core RC8–39 from the Crozet Plateau of the Antarctic Ocean (long 42°21'E, lat 42°53'S, water depth 4,330 m). The sample preparation and measurement techniques are based on those of Zimmerman<sup>2</sup> and will be described in detail elsewhere.

Figure 1a shows a typical glow curve obtained for the sample at 580 cm below the top of the core. All the other samples gave similarly shaped glow curves but with an overall increase of TL intensity with depth down the core. A convenient measure of the TL intensity is the  $\gamma$ -radiation dose,  $D$ , that produces the same TL; we determined this using the additive dose method<sup>1</sup> in which the TL of the samples is compared with the TL of identical samples which have also received a laboratory radiation dose. Figure 2a shows the value of  $D$  obtained for each sample at the 300 °C glow-curve temperature, and that  $D$  increases with depth down the core.

A similar increase of TL with depth was previously reported in two North Pacific cores by Huntley and Johnson<sup>4</sup>. They thought that the TL was coming from the separated siliceous tests; however, we have since discovered that it was coming primarily



**Fig. 1** Thermoluminescence glow curves from RC8–39 580 cm: a natural; b natural sample after a 40-min sunlamp exposure, c–e as b but after additional  $\gamma$  doses of 7.4, 14.8 and 22.2 krad respectively.



**Fig. 2** ○, The TL intensity against depth for RC8–39 evaluated at a glow-curve temperature of 300 °C.  $D$  is the  $\gamma$  dose which produces the same TL as observed in the natural samples and was obtained by the additive dose procedure. ×, Values of  $D_0$  for the same samples.

from the inorganic sediment still attached to the siliceous tests. In all three cores the TL of samples near the top of the core is significantly less than the TL of samples with an age of  $\sim 10^5$  yr. We have found a similar low TL intensity in four other core top samples we have studied. We deduce, therefore, that the TL of freshly deposited sediment is small, and not in saturation as one might expect for material derived from terrestrial sources.

Further evidence for a low TL level in freshly deposited sediment is found in the TL studies of Quaternary sediments in the USSR<sup>5–9</sup> and China<sup>10</sup>. Here an increase of TL with depth was also observed. In the original Soviet work Morozov<sup>5</sup> and Shelkopyas<sup>6</sup> suggested that the effects of sunlight and grinding during weathering were responsible for the low level of TL in freshly deposited sediments. Consequently we have examined the effects of sunlight on the TL of our samples and found a remarkable sensitivity: 20 min of exposure is enough to halve the natural TL.

To test the proposal that exposure to sunlight is a viable mechanism for setting the natural thermoluminescence to near zero we gave a set of identical samples a large  $\gamma$  dose ( $\sim 400$  krad), to bring them near saturation, and then measured the TL as a function of light exposure. This experiment was performed with a Sylvania sunlamp which is about four times as efficient as sunlight so that reproducible exposures could be obtained. The results, in Fig. 3, show that an exposure equivalent to  $\sim 10$  d of direct sunlight is capable of reducing the TL to the level found in a sample at the top of the core. Hence we suggest that previously acquired geological TL could be erased by exposure to sunlight before deposition on the ocean floor. However, we have not carried out any detailed mineral separation and chemical and mechanical alteration during weathering and sedimentation may play a significant role as well. Whatever the cause of the low TL in freshly deposited sediment the following arguments may still be applied.

As Fig. 3 shows, even after long light exposures there is a finite TL signal. (This may be due to the presence of a TL signal that cannot be bleached easily or may be due to a non-radiation-induced signal.) We therefore describe the natural TL of a sediment sample by

$$I_{\text{nat}} = I_o + I_d \quad (1)$$

where  $I_o$  is the TL at the time of deposition and  $I_d$  is the TL due to the radiation dose since deposition. Ideally a core top sample should yield a TL signal  $I_o$ . In practice, however, its TL may also be expected to have a small  $I_d$  component due to the real age of the material because of loss of the most recent sediment during coring and because of sediment mixing due to bioturbation<sup>11</sup>.

Rewriting equation (1) in terms of the  $\gamma$  doses that produce the same TL intensities we have

$$D = D_o + ED \quad (2)$$

where  $ED$ , the equivalent dose, is that laboratory  $\gamma$  dose that produces the same TL intensity as did the radiation dose experienced by the sample since deposition.

To determine  $ED$  we have developed the procedure shown in Fig. 1. All measurements are made on separate identical samples. Curve  $a$  is the TL of a natural sample. Curve  $b$  is the TL of a similar sample exposed to the sunlamp for 40 min. Curves  $c-e$  are the TL values of samples exposed to the sunlamp and then given various  $\gamma$  doses. Figure 1 shows that a  $\gamma$  dose of about 13 krad would give a TL similar to that of the natural sample above 250 °C. This dose is called  $G$ . Figure 3 shows that the 40-min sunlamp exposure was not sufficient to remove all of the  $I_d$  component of the natural TL. Let us call the fraction that remained  $f$ . Then we have

$$ED = \frac{G}{1-f} \quad (3)$$

We have determined  $f$  in separate experiments in which samples were first given long sunlamp exposures,  $\gamma$  irradiated, and then given 40-min sunlamp exposures. The values of  $f$ , typically 0.2, did not depend measurably on the  $\gamma$  dose or core sample.

We tested the above procedure for obtaining  $ED$  by using it to determine the dose for samples which had been given a known laboratory dose after a substantial sunlamp exposure. As an additional test we have taken several sets of the 119 cm samples, given them known  $\gamma$  doses,  $\Gamma$ , in addition to the natural dose, and then used the above procedure to determine  $G$  for each set. The plot of  $G$  against  $\Gamma$  was linear and when extrapolated to  $G=0$  yielded a value  $\Gamma = -ED$  independent of temperature above 250 °C and in accord with the one obtained by the

previous method. The above tests gave agreement within  $\pm 5\%$ .

The equivalent doses we have determined using equation (3) for six samples of RC8-39 are listed in Table 1. The increase with depth closely parallels that shown in Fig. 2. Values of  $D_o$  were obtained, by subtraction ( $D_o = D - ED$ ), and those at 300 °C are shown in Fig. 2; they show no systematic variation with depth and therefore are consistent with the model.

From the equivalent doses listed in Table 1 we have calculated TL ages using radiation dose rates determined by the standard techniques worked out for TL dating of archaeological material<sup>1</sup>. The age equation we have used is

$$ED = (R_K + R_U + R_{Th})T + R_1\tau\{\exp(T/\tau) - 1\} \quad (4)$$

where  $T$  is the age, the  $R$  values are the effective radiation dose rates due to the decay of  $^{40}\text{K}$ ,  $^{238}\text{U}$ ,  $^{232}\text{Th}$  and  $^{230}\text{Th}$  respectively and include  $\alpha$ ,  $\beta$  and  $\gamma$  contributions. The last term, which is dominant arises from the presence of excess  $^{230}\text{Th}$  which is continuously precipitated from the ocean; the  $^{230}\text{Th}$  mean life  $\tau$  is 108 kyr and thus it is necessary to include the variation of dose rate with time. Each term in the equation includes a water

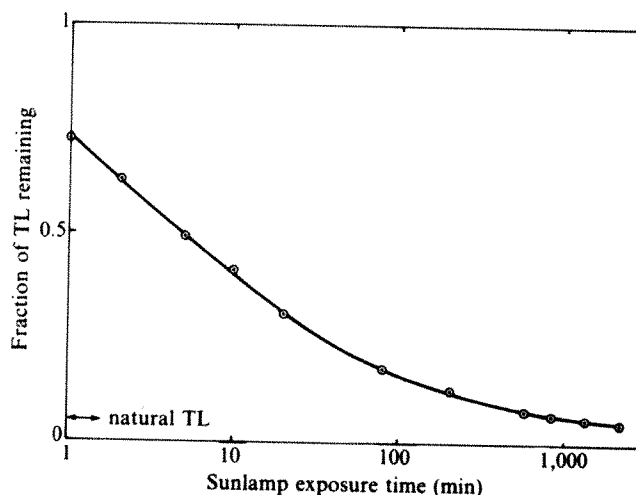


Fig. 3 The fraction of the TL evaluated at a glow-curve temperature of 300 °C remaining after a sunlamp exposure against exposure time. The experiment was performed on a set of samples which had been given a laboratory dose of 400 krad to simulate their condition before weathering.

attenuation factor calculated using an estimated water content of  $57 \pm 5\%$ , and each  $\alpha$  dose-rate term includes a measured TL efficiency factor<sup>12</sup>. The  $^{238}\text{U}$ ,  $^{232}\text{Th}$  and  $^{230}\text{Th}$  contents were determined using  $\alpha$ -scintillation counting and the dose rate conversion factors calculated according to the method described by Bell<sup>13</sup>.

The ages thus calculated are shown in Table 1 where they are compared with those we have deduced from the *Cycladophora davisiana* and oxygen-isotope variation curves of Hays *et al.*<sup>3</sup>. We consider the correlation between the two sets of dates to be sufficiently encouraging to suggest that the method is basically sound. Complete details of the measurements will be published elsewhere.

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Table 1 Doses, radioactivity analyses, TL ages and *C. davisiana* ages for RC8-39

Depth (cm)	Equivalent dose (krad)	K content (%)	Measured $\alpha$ -count rate ( $\text{ks cm}^{-2}$ ) <sup>-1</sup>	TL age (kyr)	Age from <i>C. davisiana</i> (kyr)
9	3.0	0.33	$1.83 \pm 0.03$	9	6-10
118	7.2	0.82	$1.25 \pm 0.02$	30	22-25
230	10.6	0.55	$0.98 \pm 0.02$	51	40-46
342	19.1	0.71	$1.02 \pm 0.03$	$\geq 76$	58-66
580	15.7	0.42	$0.77 \pm 0.01$	85	85-90
902	25.6	0.53	$0.56 \pm 0.01$	140	125-135

The *C. davisiana* ages were estimated by matching the *C. davisiana* variations shown in Figs 2, 3 and 5 of Hays *et al.*<sup>3</sup>. The known experimental uncertainty in the TL ages is about 18%, arising primarily from the stated uncertainty in the water content. The TL age for the 342 cm sample is shown as a lower limit because it showed anomalous fading; the others did not.

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## Albedo contrast and glaciation due to continental drift

A POPULAR theory<sup>1,2</sup> holds that glaciation occurs when landmasses drift over the poles and cool the Earth. However, the Earth cools on balance when landmasses bunch in the tropics, rather than over the polar caps. I suggest here that a peculiar distribution of land may thus be required during glacial periods, perhaps explaining their rarity<sup>3</sup> and the long intervals when land was over the poles but the Earth was not glaciated<sup>4</sup>.

The conventional argument<sup>5</sup> assumes that the Earth cools when landmasses of variable but high albedo displace water of low albedo at the poles. While snow, which has very high albedo, is more likely to accumulate on land than on water, the first snow must fall on a 'dark' landscape. A study of the atmospheric and intrinsic determinants of surface albedo, and of the best measurements, shows that the land–water albedo contrast is greatly attenuated at the poles, and indeed is greater in the tropics, for any plausible state of affairs not involving snow.

For unidirectional radiation, received directly from the Sun, the albedo of a smooth surface is inversely proportional to  $\sin E$ , where  $E$  is the angle of elevation of the Sun<sup>6,7</sup>. Under perfectly diffuse radiation, the same surface has a different albedo, not dependent on  $E$ . The surface irradiance  $Q_*$  is never unidirectional; the atmosphere scatters the direct beam from the Sun and increases the diffuse fraction  $q_d$  of  $Q_*$ , the more so as  $E$  approaches  $0^\circ$  and the path length through the atmosphere increases. Solar radiation is never perfectly diffuse, but it is virtually so when the Sun is just below the horizon, and also under low overcasts.

As well as depending on  $E$ , the reflectivity of many surfaces varies with roughness. The choppier the sea, or the taller the plants<sup>8</sup>, the less the albedo, because multiple reflection between surface elements increases the chance of eventual absorption. Multiple reflection also reduces the albedo at high  $E$ , reinforcing the theoretical dependence mentioned above. These opposing tendencies, and that of  $q_d$  to increase at low  $E$ , make empirical facts essential.

For water the data of Grishchenko<sup>7,9</sup>, for 0–25% cloud and waves of height  $<0.7$  m, are outstanding. His albedos are much lower than those predicted for direct radiation incident on a

plane, rising from 4 to 8% at large  $E$  to a peak at  $\sim 39^\circ$  around  $E = 6$ – $8^\circ$ , then dropping to about 12–14 at  $E = 0^\circ$ . These low albedos can be taken to mean that  $Q_*$  becomes almost entirely diffuse at low  $E$ . For plant albedo, the following reliable ranges (in percentage points) are available:

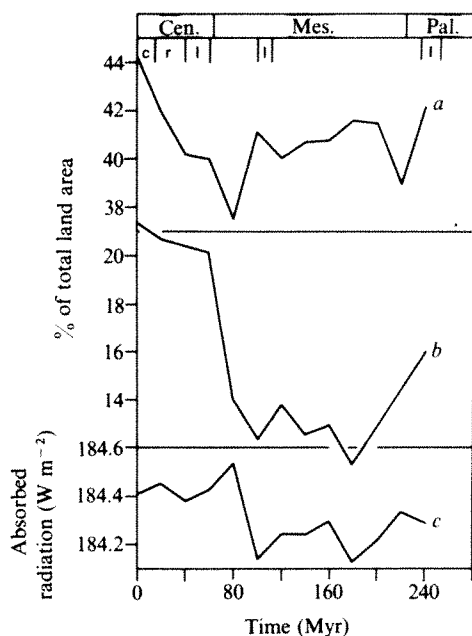
- 3 (grass,  $E = 70^\circ$  to  $15^\circ$ )<sup>10</sup>
- 8 (grass,  $E = 55^\circ$  to  $10^\circ$ )<sup>7,11</sup>
- 15 (apple tree,  $E = 80^\circ$  to  $10^\circ$ )<sup>12</sup>
- 10 (Scots pine,  $E = 65^\circ$  to  $0^\circ$ )<sup>13</sup>

Tooming<sup>11</sup> found a decrease similar to Grishchenko's below  $E \approx 10^\circ$ . It seems that plant albedo varies much less with solar elevation than does water albedo.

This implies that, when the Earth is free of snow, the land–water albedo contrast is largest in low latitudes. As  $E$  decreases polewards, high-latitude waters will have albedos considerably greater than low-latitude waters, while landmasses, presumably vegetated, will increase in albedo much less towards the poles. The best way to cool the Earth in times of benign climate is therefore to place as much land as possible in the tropics.

I have tested this proposition by planimetry of land areas in  $10^\circ$  latitude belts on palaeogeographic maps<sup>14,15</sup> at 20-Myr intervals from about 240 Myr to 0 Myr. Sea level is assumed constant. These maps suffer from doubts as to the timing and geometry of drift; placement errors of the order of  $10^\circ$  (1 latitude interval) must be suspected, although their global sum will approach zero. Near the pole, albedo changes rapidly and fine resolution is desirable. I have calculated at  $10^\circ$  resolution, but have lumped the data into tropical and polar zones which are wide relative to the possible errors.

Figure 1a shows that the tropics have become much less watery in the past 80 Myr. The Earth has grown colder throughout the Cenozoic<sup>4,16</sup>, and the cooling is thought to have accelerated, just as the calculated increase in tropical landmass has. The tropical landmass has recently reached and surpassed a



**Fig. 1** a, Landmass between  $30^\circ$  N and  $30^\circ$  S; b, landmass polewards of  $60^\circ$  N and  $60^\circ$  S (both as % of total land area  $\approx 148 \times 10^6$  km<sup>2</sup>); c, world average absorbed solar radiation (equation (1)). At top of graph c, r, l stand for continental, regional and local glaciation respectively<sup>3,16,18</sup>. The age of 240 Myr data points is merely convenient; the map is dated at  $250 \pm 25$  Myr (ref. 15), and may or may not represent the early Permian glacial period. Errors of reconstruction are discussed in ref. 14; mean palaeomagnetic poles have 95% confidence limits of from  $8.7^\circ$  to  $4.5^\circ$ ; error in planimetry (sum of  $10^\circ$ -belt measurements/ $148.0 \times 10^6$  km<sup>2</sup>) on the several maps was from  $+1.6\%$  to  $-1.6\%$ .



size last attained in the Palaeozoic. Figure 1b, for comparison, shows an increase during the Cenozoic in the land surface polewards of 60° N and 60° S. Antarctica has moved little for 80 Myr<sup>15</sup> or even 100 Myr<sup>17</sup>, but it has been glaciated only since 40 Myr, with an ice-sheet since 12–15 Myr<sup>4</sup>. North-east Siberia was over or near the North Pole throughout the Triassic and Jurassic, periods for which no one has found any glacial evidence anywhere. Ice-rafted detritus is, however, found in north-east Siberia in the late Permian<sup>18</sup>. Gondwanaland was over the South Pole until ~30 Myr after the Permo-Carboniferous glacial period ended around 260 Myr<sup>3,15</sup>.

Allowing for uncertainty, either graph might account for known climatic trends. I suggest that Fig. 1a points to a new instability of the climate, underlying the widely-accepted instability of Fig. 1b.

The new instability would work as follows. When drift concentrates land in the polar caps, the tropics get wetter. Albedo contrast produces slight polar cooling and substantial warming of the tropics. On balance, the steeper polewards temperature gradient is overshadowed by the increase in world average temperature. There may be periods, such as the early Cretaceous<sup>19</sup>, of 'stable cold' limited regionally and seasonally, but global warmth is the rule, exemplified by the Mesozoic as a whole. When landmasses drift into the tropics, the caps get wetter and slightly warmer while the tropics cool substantially. Global cooling overshadows the reduced temperature gradient, and cold periods are unstable: they are more likely to intensify by the persistence of fallen snow and, for snow to persist, dry land must be suitably placed in high latitudes. This second source of instability implies that land over the poles is necessary but not sufficient for glaciation. Figure 1a and b are thus not inconsistent. Figure 1a yields a stronger necessary condition: land concentrated in the tropics as well as near the poles. By implying watery mid-latitudes, this supports ideas of the circulation required to nourish polar glaciers<sup>4</sup>.

The significance of these landmass motions can be assessed with a simple climatic model. For each 10° belt the absorbed solar radiation is

$$Q_a = (1 - \alpha) m Q_s \quad (1)$$

where  $Q_s \propto S/4$  is the incident radiation without the atmosphere<sup>20</sup>, with the solar constant  $S = 1,361 \text{ W m}^{-2}$ .  $m = Q_a/Q_s = 0.59$  is atmospheric transparency, fixed at the modern world average<sup>21</sup>. The surface albedo  $\alpha$  is a mean of land and water albedos,  $\alpha_L$  and  $\alpha_W$ , weighted by area.

$\alpha_W$  depends on latitude as follows:

Latitude	0–50	50–60	60–70	70–80	80–90
Albedo	5	6	8	10	12

with weight given to the work of Sivkov<sup>22</sup> and Grishchenko<sup>9</sup> and to the knowledge that  $q_d$  is large under clouds<sup>23</sup>.  $\alpha_L$  was chosen to be constant at 15, close to modern albedos for temperate forests<sup>24,25</sup>, marshland<sup>24</sup>, equatorial rain forest<sup>8</sup>, wet-season savanna<sup>8</sup> and tundra in summer<sup>24</sup>. Desert<sup>24</sup>, at 17–29, is the only large terrain type brighter than 15. A constant  $\alpha_L$  may seem questionable, but the argument hinges solely on a decrease in  $(\alpha_L - \alpha_W)$  with latitude. The claim is one of logic, not of numerical realism.

Placement errors affect the calculated radiation, whether or not they sum to zero, but this uncertainty is much smaller than the observed range of  $0.41 \text{ W m}^{-2}$  (Fig. 1c). A rough numerical idea of the corresponding range of temperature  $\Delta \bar{T}$  can be obtained from the expression<sup>26</sup>  $\Delta \bar{T} = k (\Delta S/S)$ , where  $\Delta S$  is a prescribed change in the solar constant and  $k \approx 140$  for several albedo-feedback models. From equation (1), making  $\Delta S$  an independent variable,  $\Delta \bar{T} \propto \Delta Q_a \approx 0.3 \text{ K}$  for the past 240 Myr. Continental drift seems a modest governor of climatic change. However, the calculations assume no snow, which is certainly unrealistic for the Cenozoic. If there is an instability, actual insolation must deviate progressively from Fig. 1c through the past 60 Myr. The absorption calculated for the present gives, when compared with the observed<sup>21</sup>  $171.5 \text{ W m}^{-2}$ , a tempera-

ture difference of 9.5 K, agreeing well with the broad average difference inferred between the present and the Mesozoic<sup>27</sup>.

When the drift sequences of the Palaeozoic are better known, more extensive tests of this work will be possible. It may have defined a stronger necessary condition for glaciation, but not its sufficiency. The idea is more firmly grounded than galactic hypotheses<sup>28</sup>; if there is a galactic mechanism, it may modulate known tectonic processes to bring about glacial periods.

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## Hidden genetic variability in two populations of a marine mussel

ELECTROPHORESIS detects only about one third of the genetic variation present in a natural population, despite its wide application<sup>1</sup>. A combination of electrophoretic and heat denaturation techniques has revealed considerable molecular variation within single electrophoretic classes of enzymes. For example, Bernstein *et al.*<sup>2</sup> have found 1.74 more alleles at the xanthine dehydrogenase locus in the *Drosophila virilis* group than had been detected by electrophoresis alone. Further such evidence<sup>3–11</sup> has concentrated on species of *Drosophila*. I report here a study of the marine mussel *Guekensia demissa* (formerly *Modiolus demissus*) which shows that in two geographically separated populations experiencing different temperature regimes, there is an apparent correlation between the distribution of thermosensitive alleles at the phosphoglucosylase (*Pgm*) locus and environmental temperature.

Samples of *G. demissa* were collected from a salt marsh at Beaufort, North Carolina (35° N) and from the intertidal region of a beach at Stony Brook, Long Island, New York (41° N). Water temperatures in the Beaufort region can reach a summer maximum of 33 °C while in Long Island Sound they rarely reach 28 °C. Air temperatures in both regions are correspondingly higher<sup>12</sup>.

**Table 1** Distribution of genotypes and allele frequencies at the *Pgm* locus in two populations of *Guekensia demissa* from Beaufort and Stony Brook

	N*	AA	BB	Genotypes					Allele frequency				$\chi^2$
				CC	AB	AC	BC	BD	A	B	C	D	
Beaufort	103	9	22	5	29	17	20	1	0.31	0.46	0.23	0.01	0.61NS
Stony Brook	51	4	9	6	9	6	17	—	0.23	0.43	0.34	0.0	0.49NS

\* Number of individuals analysed.

 $\chi^2$ , Goodness-of-fit of observed to expected frequencies from Hardy-Weinberg equilibrium.NS,  $P > 0.05$ .**Table 2** Distribution of genotypes and allele frequencies at the *Pgm* locus in two subsamples of *Guekensia demissa* from Beaufort and Stony Brook retained for heat stability studies

	N	AA	BB	Genotype					Allele frequency				$\chi^2$
				CC	AB	AC	BC	BD	A	B	C	D	
Beaufort	39	5	8	3	10	6	6	1	0.34	0.42	0.23	0.01	1.43NS
Stony Brook	40	5	7	6	7	3	12	—	0.25	0.41	0.34	0.0	4.47NS

In *G. demissa* *Pgm* is coded at a single locus, with three common alleles *Pgm*<sup>A</sup>, *Pgm*<sup>B</sup> and *Pgm*<sup>C</sup> present in both populations, and a rare allele *Pgm*<sup>D</sup> detected only in the Beaufort sample. Two procedures were initially used to evaluate the thermostability of these *Pgm* alleles. First, two aliquots of a homogenate of adductor muscle from a single individual were incubated at two temperatures, followed by electrophoresis and staining; second, slices of a starch gel were incubated at two temperatures after electrophoresis of a homogenate of adductor muscle from a single individual, followed by the addition of *Pgm* stain. Temperature and duration of incubation were decided empirically. Heat stability studies were performed using the first procedure in preference to the second because conditions could be controlled more easily.

Table 1 presents allele frequency data for the two samples of mussels. The samples were similar in allele frequency and no significant departures from Hardy-Weinberg expectations were observed. No significant differences in either genotypic ( $P = 0.20$ ) or allelic ( $P = 0.07$ ) proportions were observed when the two samples were compared. Smaller numbers of individuals within each of these samples were used for thermostability studies and Table 2 gives allele frequency data for these subsamples. Allele frequencies were similar to those observed in Table 1, with no significant departures from Hardy-Weinberg equilibrium. In addition, no significant differences in either genotypic ( $P = 0.52$ ) or allelic ( $P = 0.28$ ) proportions were observed between the two smaller samples.

A *Pgm* band was classified as being either heat-resistant (in this case *Pgm* activity present after 15 min at 60 °C) or heat-sensitive (no *Pgm* activity after 15 min at 60 °C). Two kinds of

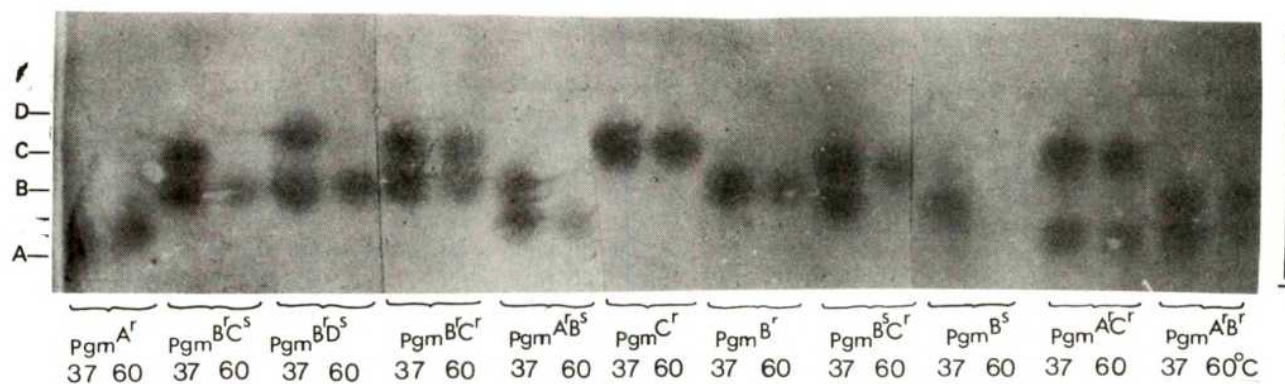
homozygotes were observed: those in which the *Pgm* band was either heat-sensitive or heat-resistant. Four kinds of heterozygotes were observed: those in which both *Pgm* bands were heat-sensitive or heat-resistant and those in which one or other of the *Pgm* bands was heat-sensitive (Fig. 1). This suggests that heat-sensitivity of each *Pgm* electrophoretic band resides at the level of the *Pgm* structural gene. Table 3 presents allele frequency data for the two populations when electrophoretic and heat-sensitivity determinations were combined. Both heat-sensitive and heat-resistant forms of the alleles *Pgm*<sup>A</sup>, *Pgm*<sup>B</sup> and *Pgm*<sup>C</sup> were observed in the two samples of mussels. In the Beaufort sample, where *Pgm*<sup>D</sup> was observed at low frequency, only the heat-sensitive form of *Pgm*<sup>D</sup> was detected. This newly discovered variation increases heterozygosity values (calculated from observed allelic frequencies) for this locus from 0.66 to 0.73 in the Beaufort sample and from 0.65 to 0.79 in the Stony Brook sample. Sixty-eight of the 78 alleles investigated in the Beaufort sample were heat-resistant and in the Stony Brook sample 56 of the 80 alleles investigated were heat-resistant. Analysis was performed on these numbers using a  $2 \times 2$  contingency  $\chi^2$  test and the distribution of heat-sensitive and heat-resistant *Pgm* alleles was found to be significantly different in the two populations ( $0.01 > P > 0.001$ ). The frequency of heat-sensitive forms of the three major alleles was consistently higher (particularly for *Pgm*<sup>B</sup>) in the sample of mussels from the cooler waters of Long Island Sound. Also, the frequency of individuals homozygous for resistant alleles was significantly greater ( $0.05 > P > 0.02$ ) in Beaufort (31 out of 39 individuals) than in Stony Brook (23 out of 40 individuals) and the frequency of individuals homozygous for sensitive alleles was greater in Stony

**Table 3** Distribution of genotypes and allele frequencies at the *Pgm* locus for two populations of *Guekensia demissa* when electrophoretic and heat sensitivity determinations are combined

	Genotypes		A <sup>s</sup>	A <sup>r</sup>	B <sup>s</sup>	B <sup>r</sup>	C <sup>s</sup>	Allele frequency		D <sup>r</sup>	$\chi^2$
								C <sup>r</sup>	D <sup>s</sup>		
Beaufort <i>n</i> = 39	A <sup>r</sup> A <sup>r</sup>	5									
	B <sup>r</sup> B <sup>r</sup>	7									
	C <sup>r</sup> C <sup>r</sup>	3									
	B <sup>s</sup> B <sup>s</sup>	1									
	A <sup>r</sup> B <sup>r</sup>	7									
	A <sup>s</sup> B <sup>s</sup>	1									
	A <sup>r</sup> B <sup>s</sup>	1									
	A <sup>s</sup> B <sup>r</sup>	1									
Stony Brook <i>n</i> = 40	A <sup>r</sup> A <sup>r</sup>	4									
	B <sup>r</sup> B <sup>r</sup>	4									
	C <sup>r</sup> C <sup>r</sup>	5									
	A <sup>s</sup> A <sup>s</sup>	1									
	B <sup>s</sup> B <sup>s</sup>	3									
	C <sup>s</sup> C <sup>s</sup>	1									
	A <sup>r</sup> B <sup>r</sup>	4									
	A <sup>s</sup> B <sup>r</sup>	3									

Superscript s and r correspond to heat-sensitive and heat-resistant forms of *Pgm* alleles.





**Fig. 1** Heat sensitivity of *Pgm* alleles in *Guekensia demissa*. (Results of two gels from the same electrophoretic run; gel papers were divided and transposed to eliminate repetitive patterns.) The posterior adductor muscle was excised from living individuals and homogenised manually in approximately two volumes of 50 mM Tris-HCl buffer, pH 7.8 (1:1 w/v). Homogenates were centrifuged at 3,500 r.p.m. for 15 min at 4 °C. The supernatant solution was divided into four aliquots, two of which were incubated in a water bath for 15 min, one at 37 °C and the other at 60 °C. The remaining two aliquots were retained for replication; hence each individual was classified on the basis of two replicated experiments. Electrophoresis was performed at 2 °C in horizontal starch gel (Electrostarch) using the Tris-EDTA-maleic buffer system, pH 7.4, of Spencer *et al.*<sup>13</sup>. Gels were run for 14–16 h at 6.5 V cm<sup>-1</sup>. Sliced gels were stained for *Pgm* using the paper overlay method described by Scopes<sup>14</sup>. The terminology used for allozymes of *Pgm* is as follows: alleles are numbered A to D in order of increasing anodal mobility. For comparative purposes individuals from both populations were run on the same gel.

Brook (7 out of 40 individuals) than in Beaufort (2 out of 39), although this difference is not significant at the 5% level ( $0.10 > P > 0.05$ ).

In spite of increasing experimental evidence for hidden genetic variability within electrophoretic allozyme classes—at least for species of *Drosophila*—there have been no reports to date on the possible biological significance of this new-found structural variability. Some workers<sup>2,5</sup> commenting on the random distribution patterns of some of the heat-sensitive allozymes of xanthine dehydrogenase and octanol dehydrogenase in the *Drosophila virilis* group concluded that such patterns support the neutral and not the selection hypothesis of genetic variability in natural populations. It is possible that the apparent non-random distribution of thermosensitive alleles in the mussel populations investigated in the present study is being maintained through selective forces acting at the *Pgm* locus. Trippa *et al.*<sup>9</sup> have suggested 'that selective values of allelic proteins with different thermosensitivities might in fact be affected by differences in the habitat', perhaps in this case temperature, an important component of an organism's environment. However, before firm conclusions can be drawn regarding the adaptive role of hidden genetic variation within populations of *G. demissa*, detailed biochemical investigations of the *Pgm* system are necessary. Also, breeding data—at present unavailable—would clarify whether the property of thermosensitivity is associated with the *Pgm* locus itself or whether it is associated with an alternative polymorphic locus.

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## Mutation at H-2K locus influences susceptibility to autoimmune thyroiditis

GENES in the major histocompatibility complex (MHC) have been found to be associated with the development of autoimmune diseases, including spontaneous diseases in man<sup>1,2</sup> and spontaneous<sup>3</sup> or experimentally induced autoimmunity in animals<sup>4</sup>. Experimental autoimmune thyroiditis (EAT) can be induced in susceptible strains of mice, such as those with the MHC H-2<sup>k</sup> haplotype, by injecting them with mouse thyroglobulin together with complete Freund's adjuvant<sup>5</sup>. In contrast, injection of mice homozygous for the H-2<sup>b</sup> haplotype fails to induce the mononuclear cell infiltration of the thyroid gland characteristic of EAT. We report here that susceptibility to induction of EAT results from an apparent point mutation which evidently occurred at the H-2K locus of the resistant H-2<sup>b</sup> haplotype. This indicates that the H-2K glycoprotein can serve to regulate the autoimmune response to thyroglobulin.

Table 1 shows the results of injecting mouse thyroid extract in adjuvant into mice of strains C3H/eb (H-2<sup>k</sup>), C57BL/6 (H-2<sup>b</sup>) or its HZ1 mutant (B6.C-H-2<sup>ba</sup>)<sup>6,7</sup>. C3H/eb mice demonstrated the response of high-responder H-2<sup>k</sup> strains with about 80% incidence of development of EAT. Only about 20% of C57BL/6 mice developed lesions of EAT, as expected for low responder mice of H-2<sup>b</sup> strains<sup>5,8</sup>. However, the HZ1 (H-2<sup>ba</sup>) mutant showed an incidence of EAT (79%) comparable with that of high-responder H-2<sup>k</sup> mice. Hence, the HZ1 mutation led to susceptibility to EAT in mice whose original genome coded for resistance.

The immunogen used in these experiments was thyroid extract obtained from C3H/eb mice. Mouse strains differ in the immunogenicity of their thyroglobulin<sup>9</sup> and we have found that thyroid extract from C3H/eb mice is strongly immunogenic whereas that of both C57BL/6 and HZ1 mice is poorly immunogenic for either high- or low-responder strains of mice (in preparation). These findings suggest that the increased



**Table 1** Susceptibility to EAT of the HZ1 mutant (H-2<sup>ba</sup>), and H-2<sup>b</sup> low- and H-2<sup>k</sup> high-responder strains of mice

Mouse strain	H-2 haplotype	Injection of thyroid extract plus adjuvant	Incidence of EAT	Antibody titre (log <sub>2</sub> )
C3H/eb	k	Yes	82% (46/56)	5.0
C57BL/6	b	Yes	20% (10/49)	5.0
HZ1	ba	Yes	79% (11/14)	7.0
		Adjuvant alone	0 (0/5)	<1.0

Mice were injected twice subcutaneously at 7-d intervals with thyroid extract emulsified in complete Freund's adjuvant, as described by Twarog and Rose<sup>21</sup>. The extract was prepared from thyroid glands of C3H/eb mice. Each mouse received the equivalent of one thyroid gland. The adjuvant<sup>21</sup> was prepared by adding 7 mg ml<sup>-1</sup> of *Mycobacterium tuberculosis* H37Ra (Difco) to incomplete Freund's adjuvant (Difco). The animals were killed 5 weeks after the second injection and histological sections of the thyroid glands were examined in a blind fashion by two independent observers. A thyroid gland was considered to be positive for EAT if it showed at least one unequivocal focal infiltrate of mononuclear cells<sup>9</sup>. Antibodies to purified thyroglobulin, prepared as described by Tomazic and Rose<sup>9</sup>, were measured in the pooled serum of mice in each group, using a haemagglutination of formalinised tanned sheep red blood cells<sup>22</sup> coated with thyroglobulin. The results represent three separate experiments. Difference in incidence of EAT between C57BL/6 and HZ1 was highly significant by the  $\chi^2$  test ( $P < 0.0003$ ).

incidence of EAT in the HZ1 mutant mice was not due to a change in the immunogenicity of their thyroglobulin.

Note that all groups developed antibodies to purified thyroglobulin after injection with thyroid extract in adjuvant. This confirms the observation that mice develop specific antibodies to determinants of thyroglobulin after injection with extract, whether or not they are genetically susceptible to histological EAT<sup>10</sup>.

The HZ1 mutant has been studied in several laboratories<sup>7,11-14</sup> and it is generally concluded that a point mutation occurred at the H-2K locus of the C57BL/6 genome. This conclusion is supported by the finding of differences in the peptide map of the H-2K glycoprotein of the HZ1 mutant, compared with the wild-type C57BL/6 strain<sup>15</sup>. This molecular difference would seem to account for the mutual T-cell reactivity of C57BL/6 and HZ1 (refs 13, 16). However, the HZ1 mutant seems to preserve other determinants on the H-2K molecule which are identical to those of the H-2K<sup>b</sup> wild type<sup>7,12</sup>.

There is no evidence of any differences between C57BL/6 and HZ1 in the I region to the right of the H-2K locus, as the immune response (Ir) genes which functionally define the I region are the same in the mutant and wild-type mice<sup>17</sup>. Thus, it seems very likely that the only genetic differences between the wild-type C57BL/6 and the HZ1 mutant mouse are at the H-2K locus of the MHC<sup>7</sup>. Hence, our results indicate that the H-2K glycoprotein can regulate the pathological expression of EAT following an autoimmune response to thyroglobulin. This implies a major role for cytotoxic T cells in EAT. Furthermore, the limited portion of the H-2K<sup>b</sup> gene in which the mutation occurred, the Z1 locus<sup>6</sup>, seems to be critical in determining susceptibility to EAT.

Previously, H-2K or H-2D gene products have been observed to restrict the cytotoxic effects of T lymphocytes against target cells infected with certain viruses<sup>18</sup>. T lymphocytes obtained from mice immunised against viruses will often kill virus-infected target cells only when the target cells and the lymphocytes have H-2K or H-2D genes in common. Note that HZ1 and C57BL/6 differ in the specificity of virus-H-2K-associated cytotoxicity<sup>13</sup>. It is thought that associative recognition by T lymphocytes of viral antigens together with MHC gene products is based on the molecular association of viruses with MHC gene products at the surface of the infected cell<sup>19,20</sup>. It remains to be determined whether susceptibility to EAT also

involves physical association between H-2K glycoprotein and thyroglobulin on the surface of thyroid epithelial cells. Such an association could occur if MHC gene products served to control movement of macromolecules across cell membranes.

The different susceptibilities to induction of EAT of the C57BL/6 strain and its HZ1 mutant suggest that these mice may be helpful in determining how MHC gene products regulate immune responses. Any differences between the mice in the handling or presentation of thyroglobulin by macrophages, lymphocytes or other cells could be related to the specific modification of the H-2K glycoprotein. Furthermore, isolation of purified H-2K glycoproteins may allow study of the interaction of these molecules with specific determinants of thyroglobulin.

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## A novel subset of antigenic cells triggers B-cell responses to MHC antigens

THE exceptional immunogenicity of major histocompatibility complex (MHC) antigens is well known but poorly understood. As there is evidence that MHC products are intimately involved in the presentation of antigen to T cells<sup>1,2</sup> it seems reasonable to expect that antigenic variants of these 'presenting structures' should be very immunogenic for T cells. However, because MHC antigens on non-nucleated cells and in subcellular forms are weak T-cell immunogens and may even function as tolerogens<sup>3</sup>, it has been postulated that an additional signal from living antigenic cells is needed to stimulate the immune response<sup>4</sup>. The response of B cells to MHC antigens is far less well understood but again an additional signal from antigenic cells seems to be needed in some primary responses, as antigenic cell fragments are not immunogenic<sup>5</sup>. We report here that in studies of primary antibody responses to H-2-incompatible cells, immunogenicity is almost completely restricted to a small, novel subpopulation of cells found in the spleen and bone

**Table 1** For induction of primary anti-H-2 PFC responses conventional T-cell competence in donors and recipients is not required

Experiment	Recipient strain	Donor strain	Dose of donor spleen cells	PFC per spleen Mean (s.e.m.)
1	CBA	B10 <i>nu/+</i> and <i>+/+</i>	$2 \times 10^6$	936 (155)
	CBA	B10 <i>nu/nu</i>	$2 \times 10^6$	2,136 (139)
2	<i>nu/+</i> and <i>+/+</i>	B10 <i>nu/nu</i>	$10^6$	1,800 (278)
	<i>nu/nu</i>	B10 <i>nu/nu</i>	$10^6$	2,730 (449)
3	<i>nu/+</i> and <i>+/+</i>	BALB/c	$10^7$	14,866 (4,050)
	<i>nu/nu</i>	BALB/c	$10^7$	8,370 (2,773)
4a	<i>nu/nu</i>	BALB/c <i>nu/nu</i>	$2.3 \times 10^6$	40,577 (8,452)
	<i>nu/+</i> and <i>+/+</i>	BALB/c <i>nu/nu</i>	$2.3 \times 10^6$	10,450 (3,215)
4b	<i>nu/nu</i>	BALB/c <i>nu/+</i> and <i>+/+</i>	$2.3 \times 10^6$	26,717 (6,331)
	<i>nu/+</i> and <i>+/+</i>	BALB/c <i>nu/+</i> and <i>+/+</i>	$2.3 \times 10^6$	21,008 (4,944)

Anti-H-2<sup>b</sup> responses were determined in experiments 1 and 2 and anti-H-2<sup>d</sup> responses in experiments 3 and 4. PFC were assayed by the technique developed by Fuji *et al.*<sup>6</sup> and modified from our method for assay of anti-Thy-1 PFC<sup>12</sup>. Briefly, 50  $\mu$ l of appropriate concentrations of spleen cells in Eagle's modified minimal essential medium (MEM) containing 10% heat-inactivated newborn calf serum (MEM+CA<sub>10</sub>) were mixed with 20  $\mu$ l of 20–40% (packed volume) of appropriate target cells. The suspension was warmed to 37 °C and 180  $\mu$ l of 1% agarose in MEM-CA<sub>10</sub>, kept at 45–48 °C, was added immediately before pouring on to 30-mm Petri dishes. These plates were incubated at 37 °C in a humidified 10% CO<sub>2</sub> atmosphere for 3.5 h, and then an anti-mouse Ig, rabbit antiserum (0.5 ml at 1:10) was added. Following a further incubation at 37 °C for 30 min 1.0 ml of selected rabbit complement at 1:15 dilution (in 10% hypotonic MEM-CA<sub>10</sub> containing 3 mM barbituric acid) was added. After 45 min incubation with complement, these plates were stained with 0.2–0.3% of Trypan blue in phosphate-buffered saline for 15 min. The numbers of plaques were counted with the aid of a dissecting microscope. Almost all PFC detected by this technique were IgM-secreting cells, as addition of a specific anti- $\mu$  serum (20  $\mu$ l of 1:60 dilute antiserum) into the mixture of spleen cells and target cells before incubation inhibited the development of plaques. Primary PFC responses assayed on day 5 are shown.

marrow. Furthermore, as the response we have studied is independent of T cells, these stimulating cells seem to be unique (perhaps specialised?) in their ability to present surface-borne antigen and the additional signal to responding B cells.

The experimental system was developed from the anti-H-2 direct plaque-forming cell (PFC) assay of Fuji *et al.* in which specificity for H-2K and H-2D region antigens has been established and other parameters of response have been defined<sup>6,7</sup>; these findings have since been confirmed in our laboratory (in preparation). Primary responses to H-2 antigens were induced by intraperitoneal injection of spleen cells into appropriate recipients. Spleens of responding mice were removed after 5 days, at the peak of response, and their content of PFC determined in the plaque assay using the lymphoma lines L1210(H-2<sup>d</sup>) or EL-4G<sup>-</sup>(H-2<sup>b</sup>) as targets.

To examine the role of T cells in the response, normal or T-cell deficient mice were immunised with antigenic spleen cells from normal or T-cell deficient donors. The response of CBA mice to spleen cells from C57BL/10 or C57BL/10 *nu/nu* donors (H-2<sup>b</sup>) shows that T-deficient cell populations may in fact provoke stronger responses than C57BL/10 *nu/+* control donor mice, which have a normal T-cell compartment (Table 1). Thus, T cells in the inoculum are apparently unnecessary for the induction of primary PFC in these conditions. Using outbred *nu/nu* and littermate *+/+* and *nu/+* recipients in combination with C57BL/10 *nu/nu* donors it is apparent (Table 1) that T-cell deficient recipients respond equally well or better than their controls to C57BL/10 *nu/nu* donors. Similarly, the response to H-2<sup>d</sup> antigens is also substantially independent of T cells in the donor or in responding mice (Table 1, experiments 3, 4). Thus, conventional T-cell functions seem to have no role in the primary PFC response to H-2 antigens.

These results significantly strengthen previous evidence that primary responses to MHC antigens of mice (and rats) are mainly independent of conventional T-cell activity<sup>8,9</sup>. It is, however, unexpected that glycoproteins such as MHC antigens

should induce substantial T-cell independent responses. First, their physical properties are at marked variance with properties common to other T-cell independent antigens, such as a polymeric molecular structure and slow catabolism *in vivo*<sup>10</sup>. Second, and in contrast to H-2, we have shown that the primary PFC response to another glycoprotein surface antigen, Thy-1, is dependent on T-cell mediated helper effects produced by other surface antigens coded elsewhere in the donor genome<sup>11–13</sup>. Taken together, these apparently contradictory observations suggest that the molecular and antigenic properties which determine the immunogenicity of cell-surface antigens cannot be predicted from present knowledge and are likely to be quite different from those for molecular antigens in solution.

In further experiments, to be reported in detail elsewhere, we found that some lymphoid tissues were markedly more immunogenic than others (for example, spleen cells are approximately 20 times more active at stimulating PFC than lymph node cells, and unfractionated peritoneal cells are virtually inactive). However, the density of H-2 antigens as determined by absorption tests was indistinguishable in the different cell populations. We therefore postulated that immunogenicity for primary PFC may not be related simply to the amount of H-2 antigen on the donor cell inoculum but could reside in an immunogenic subpopulation of cells which is present in higher frequency in the spleen. To examine this possibility donor spleen cells were fractionated with respect to (1) adherence to nylon

**Table 2** Characterisation of spleen cells immunogenic for primary anti-H-2 PFC

Experiment (recipient strain)	Donor pre-treatment	Fractionation Adherence	Density	Cell recovery (%)	Dose of viable cells	PFC per spleen
1. (CBA)		Unseparated		100	$2 \times 10^6$	2,136
		Adherent		31 (55)	$2 \times 10^6$	900
		Non-adherent		22 (45)	$2 \times 10^6$	5,028
2. (CBA)		Unseparated			$1 \times 10^6$	1,356
		Non-adherent			$1 \times 10^6$	1,548
		Non-adherent	Low†		$5 \times 10^5$	0
		Non-adherent	Low†		$5 \times 10^6$	0
		Non-adherent	High†		$5 \times 10^5$	1,608
3. (CBA)		Non-adherent			$1 \times 10^5$	0
		Non-adherent			$5 \times 10^5$	348*
		Non-adherent	Low	62 (85)	$5 \times 10^5$	0
		Non-adherent	Low		$5 \times 10^6$	0
		Non-adherent	High		$1 \times 10^5$	120*
		Non-adherent	High	11 (15)	$5 \times 10^5$	1,425
4. (C57 BL/10)		Adherent			$1 \times 10^5$	225*
		Non-adherent	Low		$1 \times 10^5$	0
		Non-adherent	Low		$2 \times 10^6$	20*
		Non-adherent	High		$1 \times 10^5$	32,364
5. (CBA)		Normal spleen			$1 \times 10^5$	75*
		Normal spleen			$1 \times 10^6$	9,945
		Normal spleen			$1 \times 10^7$	7,704
		Irradiation Unseparated		8	$5 \times 10^4$	15*
		Irradiation Unseparated			$1 \times 10^5$	7,632
		Irradiation Adherent			$1 \times 10^5$	1,020
		Irradiation Non-adherent	Low		$1 \times 10^5$	0
		Irradiation Non-adherent	High		$5 \times 10^3$	315
		Irradiation Non-adherent	High		$5 \times 10^4$	13,812

The results show that the immunogenicity belongs to a small subset of cells which is relatively non-adherent to nylon wool, of unusually high density, and resistant to irradiation administered *in vivo*. Donor BALB/c mice were irradiated at 900 R from a <sup>60</sup>Co source 20 h before removal of spleens. For fractionation, 1 ml of an appropriate concentration ( $5 \times 10^7$  ml<sup>-1</sup>) of spleen cells from normal or irradiated BALB/c mice in MEM containing 5% fetal calf serum (MEM-F<sub>5</sub>) were loaded on nylon wool columns each of 0.6 g in a 6-ml volume. Nylon-non-adherent cells were eluted after incubation of the columns at 37 °C for 1 h by the procedure of Julius *et al.*<sup>22</sup>. Nylon-adherent cells were also collected after thoroughly washing the columns according to the method of Trizio and Cudkovic<sup>23</sup>. Five ml of the suspension of nylon-non-adherent cells ( $< 10^7$  p/ml<sup>-1</sup>) were layered on 3 ml of Ficoll-Hypaque solution ( $\rho = 1.082$ ) and centrifuged at 3,000 r.p.m. for 20 min at 20 °C. The pellet fraction (high-density fraction) and interface fraction (low-density fraction) were collected and washed three times with MEM at 150 g for 8 min. Day 5 primary PFC responses are shown. Each value represents the mean of five mice. The standard error of each value is less than 25%, except the values marked with an asterisk ( $\geq 25\%$ ). Donor cells for experiment 4 were irradiated *in vitro* at 2,000 R before injection. Cell recovery is given as the percentage of recovery of cells in each fraction to overall recovery of cells after fractionation.

† Treated with 0.83 M ammonium chloride to lyse erythrocytes before injection.

**Table 3** Ability of spleen cell fractions to stimulate PFC to H-2 antigens does not correlate with their stimulatory ability in MLC

Cell fraction	Buoyant density	MLC		PFC per spleen
		c.p.m.	Index	
Nylon-adherence				
Original		65,112	5.3	
Adherent	—	45,748	3.7	225 (61)
Non-adherent	—	29,040	2.3	
Non-adherent	Low	43,972	3.6	0 (0)
Non-adherent	High	7,854	-0.6	32,364 (6,990)
(Syngeneic control)		12,264	1.0	

For MLC,  $6 \times 10^5$  CBA spleen cells in 0.1 ml of RPMI medium containing 10 mM HEPES,  $5 \times 10^{-5}$  M 2-mercaptoethanol and 5% heat-inactivated fetal calf serum were mixed with  $6 \times 10^5$  CBA spleen cells (syngeneic control) or BALB/c spleen cells which had been fractionated and irradiated at 2,000 R. They were incubated in a humidified, 10% CO<sub>2</sub> atmosphere at 37 °C for 5 d, and then pulsed with 1  $\mu$ Ci of [<sup>125</sup>I]5-iodo-2'-deoxyuridine. After 4 h incubation the cultures were collected on filter paper for counting. MLC results are the mean of three to four cultures. PFC results are day 5 primary anti-H-2 PFC responses of B10 mice injected with  $10^5$  cells. This is part of experiment 4 in Table 2. Values represent mean PFC (s.e.m.).

wool, (2) buoyant density and (3) resistance to  $\gamma$ -irradiation administered *in vivo* 1 day before the donors were killed. It is clear from results using nylon wool columns (Table 2, experiments 1, 2, 4, 5, and Table 3) that on comparing inocula of viable cells, the immunogenic cells are enriched in the non-adherent fraction and significantly depleted in the adherent fraction. Subsequent fractionation of the non-adherent cells on Ficoll-Hypaque (density 1.082 g cm<sup>-3</sup>) showed that immunogenic cells are enriched in the dense fraction (the pellet) and virtually absent in the lymphoid cell-rich, interface buoyant fraction (Table 2, experiments 2-5, and Table 3).

The radiosensitivity of the immunogenic cell fraction was assessed by irradiation of donors with 900 R from a <sup>60</sup>Co source 20 h before removal of their spleens. It is evident (Table 2, experiment 5) that spleen cells from irradiated donors are considerably more immunogenic than similar numbers of normal spleen cells. Further fractionation of this irradiated population of nylon wool columns resulted in decreased immunogenicity in the adherent cell fraction, as before. The non-adherent fraction was separated on Ficoll-Hypaque and showed, also as before, no immunogenicity of the interface cells but very high immunogenicity of the dense fraction. The factor of enrichment for the subpopulation of immunogenic cells with the three procedures in combination is estimated to be 50- to 70-fold. The data presented above, together with the unusual tissue distribution of the immunogenic cells, their presence in nude mice, the results of the fractionation procedures and some unpublished results, make it unlikely that the immunogenic cells are conventional T, B or macrophage-like cells. Furthermore, cells of the granulocytic series which are usually enriched in high-density fractions are highly radiosensitive and adherent to nylon, as confirmed by microscopic observation.

Recent evidence indicates that specialised accessory cells are highly efficient in the presentation of antigen to T cells<sup>14</sup>, and these cells are the most active in stimulating allogenic responses in mixed leukocyte cultures (MLC)<sup>15</sup>. As the MHC antigens on the non-adherent, dense cells stimulated B cells to produce PFC in our experiments, we also examined the ability of these cells to stimulate T cells in MLC<sup>16</sup>. As shown in Table 3 no correlation was observed between cell fractions which stimulated in MLC and those which stimulated PFC in the same experiment. In fact, the non-adherent high-density fraction, which is by far the most effective in inducing PFC, reproducibly induced little or no MLC. This result, taken with the other physical parameters considered above, seems to distinguish the novel PFC-stimulating fraction from the cells considered to be functionally specialised for antigen presentation to T cells<sup>14,15,17</sup>.

The normal role of these B-stimulating cells in the immune system is unknown but their unusual properties should facilitate investigation. However, *in vitro* studies show that accessory cells or antigen-presenting cells are involved in B-cell triggering for

T-cell dependent<sup>18</sup> and possibly some T-cell independent responses<sup>19-21</sup>. Such cells may be identical to the antigen-presenting cells for T-cell responses or may comprise a new subpopulation of cells. The novel subset of cells reported here to be exceptionally immunogenic for B cells may have a role in antigen presentation and B-cell triggering.

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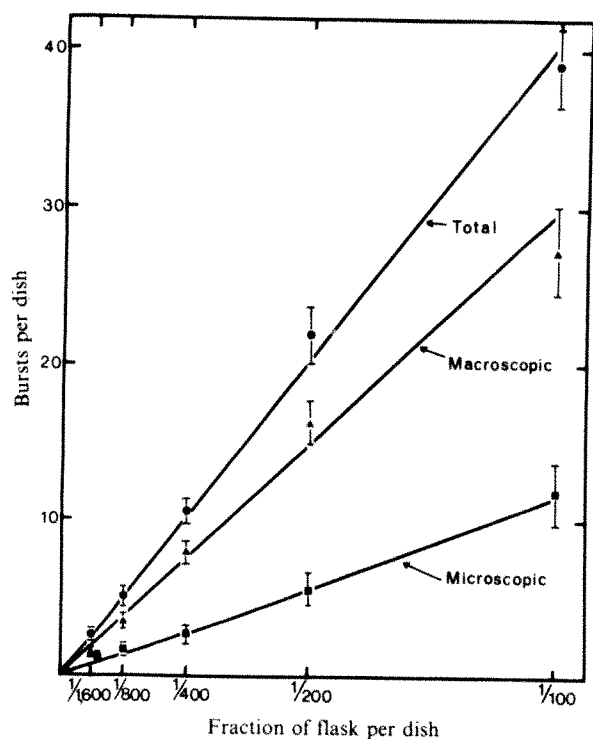
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## CFU-S in individual erythroid colonies derived *in vitro* from adult mouse marrow

It is generally accepted that in the haematopoietic system there is a hierarchy of pluripotent and committed progenitor cell types. Representative committed progenitors from all the myeloid and lymphoid pathways can be detected by *in vitro* colony assays<sup>1</sup>. This methodology has been extended to include multipotent haematopoietic progenitors<sup>2-5</sup>, and it has since been shown that at least one such multipotent cell type, the progenitor of macroscopic erythroid-megakaryocyte bursts, undergoes self-renewal during colony formation *in vitro*<sup>5</sup>. We now report that cells capable of macroscopic spleen colony formation (CFU-S)<sup>6</sup> are present in macroscopic bursts and that on average two to three and up to five self-renewal divisions of CFU-S can occur during the first 9 d of burst growth. These findings raise the possibility that clonal analysis techniques<sup>7</sup> may be combined with cell culture manipulations to investigate how the choice between self-renewal and progressive differentiation is determined.

Macroscopic bursts were generated in standard methylcellulose cultures containing 2.5 units ml<sup>-1</sup> erythropoietin (Connaught, Step III) and pokeweed mitogen-stimulated spleen cell conditioned medium (final concentration 0.3%) as previously described<sup>5</sup>. These conditions are optimal for macroscopic burst formation and give a linear relationship between





**Fig. 1** Burst formation as a function of the number of cells plated. Cells were pooled from three replicate 2-week-old flask cultures maintained and assayed as described in the text. The number of cells in each assay dish is expressed as a fraction of the total number of non-adherent cells collected per flask. In this experiment 1/100th of a flask contained  $6.4 \times 10^4$  cells. Values shown represent the mean count  $\pm 1$  s.e.m. for a minimum of four assay replicates.

burst number and cell concentration (Fig. 1). They are inadequate for significant granulocyte colony formation. The cells used for plating were from the non-adherent cell fraction of 2-week-old flask cultures prepared using adult B6C3F<sub>1</sub> mouse marrow cells as previously described<sup>8,9</sup>. Although cells capable of macroscopic burst formation are present in fresh as well as cultured adult marrow, their incidence in fresh marrow is much lower (only 5–10 per  $10^5$  nucleated cells) and they constitute less than one-third of the total late-maturing burst progenitor population<sup>5</sup>. In the conditions of flask culture used here, there is a preferential proliferation and survival of macroscopic burst progenitors so that by 2 weeks their concentration reaches 30–50 per  $10^5$  nucleated cells and they constitute the predominant erythropoietic cell type<sup>5,8</sup>. Thus, by plating  $2.5 \times 10^4$  flask cultured cells (that is, 1/200th of a flask, washed and diluted in  $\alpha$ -medium plus 2% fetal calf serum) 10–20 well isolated macroscopic bursts were obtained in each assay dish (Fig. 1), and the problem of contaminating background colonies derived from either granulopoietic progenitors (CFU-C) or more mature erythropoietic progenitors (day 14 BFU-E micro, day 3 BFU-E, CFU-E) was also eliminated. After 9 d of incubation, macroscopic bursts could be readily identified as red spots visible to the unaided eye (Fig. 2a). Such bursts were picked and assayed for CFU-S (see Table 1 legend for details). Macroscopic spleen colony counts were carried out on day 9 (Fig. 2b).

Of 25 macroscopic bursts assayed individually, 11 (44%) produced spleen colonies (Table 1a). On average each produced one spleen colony although values of up to four were obtained. The incidence of approximately one CFU-S per macroscopic burst was confirmed when suspensions consisting of four or eight pooled macroscopic bursts were assayed, and the proportion of pools that were positive increased to 78% and 87%, respectively. The wide distribution of values obtained for different pools of four and even eight bursts also confirms the heterogeneity seen when individual bursts were assayed. Presumably, some of the macroscopic bursts contained at least four times the overall average number of CFU-S. Using an estimate of 0.15 for

the seeding fraction ( $f$ )<sup>10</sup>, the total CFU-S per macroscopic burst was on average 6.7 and must have reached at least 27 in some bursts. This would indicate an average of two to three, and up to five self-renewing cell divisions.

Spleen colony formation was not associated with a cell type that occurred randomly throughout the burst assay culture, for aliquots of 'background' culture equivalent to three times the culture volume contained in a pool of eight macroscopic bursts did not produce spleen colonies (Table 1a). Irradiation of the injected cells also eliminated spleen colony formation (Table 1b). In addition, karyotype analysis of metaphases in female recipient spleens removed 10 d after irradiation and injection with male macroscopic bursts showed 33 of 37 metaphases to be of male, that is, burst cell, origin (of the remaining four metaphases, three were scored as probable males and one as female). For this analysis three spleens with visible surface colonies were made into single cell suspensions 1–2 h after intraperitoneal injection of 50  $\mu$ g colcemid. Metaphase spreads were C or trypsin G-banded and a sample of technically suitable metaphases photographed and evaluated by P.J. and F.D. for 40 chromosomes and the Y chromosome<sup>11</sup>. These results indicate that the spleen colonies obtained resulted from proliferation of cells originally present in macroscopic bursts.

Previous experiments with macroscopic bursts have suggested a close relationship between their progenitors and CFU-S<sup>5</sup>. The present findings provide direct evidence for overlap between the cell populations detected by these two assays. Further experiments will be required for full characterisation of the *in vivo*

**Table 1** Number of spleen colonies produced by samplings from macroscopic burst assays

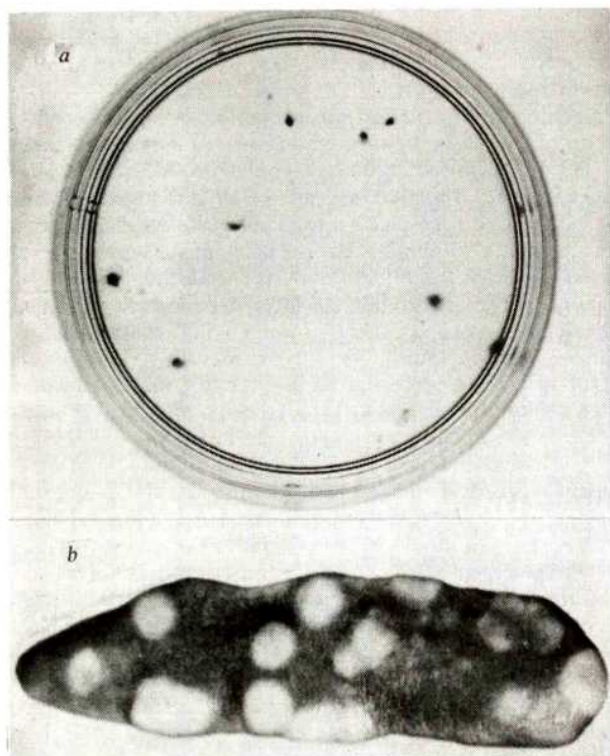
a Macroscopic burst cells vs surrounding culture medium (background)				
	1 Macro burst per recipient	4 Macro bursts per recipient	8 Macro bursts per recipient	Background
Exp. 1	2, 0, 4, 2, 0	13, 1, 0, 1, 17	4, 13, 1, 2, 3	0, 0, 0, 1
Exp. 2	2, 0, 0, 1, 1	3, 1, 0, 6, 0	12, 2, 5, 22, 0	0, 0, 0, 0, 0
		0, 3, 4, 5		0, 0, 1
Exp. 3	ND	4, 7, 2, 7	ND	0, 2, 0, 0
Exp. 4	0, 0, 0, 0, 1	ND	0, 20, 2, 10, 8	0, 1, 1, 1, 0
	3, 0, 3, 0, 0,			
	4, 1, 0, 0, 0			
CFU-S per pool (mean $\pm$ s.e.m.)	1.0 $\pm$ 0.3	4.1 $\pm$ 1.1	6.9 $\pm$ 1.8	0.3 $\pm$ 0.1
CFU-S per macro burst	1.0	1.0	0.9	—
b Non-irradiated vs irradiated macroscopic burst cells				
	Non-irradiated		Irradiated	
Exp. 5 <i>In vivo</i> irradiation	6, 2, 2, 2, 19, 22, 27		0, 0, 0, 0, 0, 0, 0	
Exp. 6 <i>In vitro</i> irradiation	5, 2, 1, 2, 4		0, 1, 1, 0, 0, 0	

Using an inverted microscope, 9-d-old isolated macroscopic bursts were selected and taken up individually into a sterile, finely drawn out Pasteur pipette containing a small quantity of  $\alpha$ -medium. Care was taken to remove each burst in the smallest volume possible (by weighing dishes this was determined to be no more than 1/250th of the total 1.0-ml culture volume) to minimise further any possible contamination with extraneous cells. The required number of macroscopic bursts were placed in a small volume of  $\alpha$ -medium on ice and a single cell suspension obtained by gentle aspiration through a no. 21 needle before injection through a no. 26 needle. B6C3F<sub>1</sub> recipient mice were given 850 rad whole-body radiation<sup>5</sup> followed by up to 0.6 ml of test material injected into the tail vein. For background values aliquots containing 10% of the culture volume (0.1-ml volumes) were picked at random under microscopic visualisation avoiding only macroscopic bursts and other large colonies ( $>100$  cells). This volume represents 3 $\times$  that picked for assays of eight macroscopic bursts and 24 $\times$  that picked for assays of single bursts. S.e.m.s were calculated by pooling data from expts 1–4. b In exp. 5, 8 separate pools, each containing 16 macroscopic bursts, were prepared. Each pool was then subdivided equally between two mice. One of these had already been given 850 rad whole-body X-irradiation before injection in the normal fashion. The other was not irradiated until immediately after injection so that in this group both the cells to be assayed and the recipients were exposed to the same 850-rad treatment. In exp. 6, 4 separate pools, each containing 16 macroscopic bursts, were prepared. Half of each pool was divided and assayed for CFU-S in two recipients in the usual fashion. The other half of each pool received 1,000 rad (X ray) *in vitro* before being divided and assayed for CFU-S in two recipients. ND, not determined.

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**Fig. 2** *a*, Gross appearance of macroscopic bursts from 2-week flask cultured marrow seen in assay dishes after 12 d incubation (2X). *b*, Gross appearance of spleen (fixed in Tellyesniczky's solution) showing colonies produced 9 d after injection of eight macroscopic bursts (5X).

repopulating and differentiative potentialities of cells derived from macroscopic burst progenitors.

The broad distribution of CFU-S numbers in individual bursts is of interest, as it approaches that observed for CFU-S self-renewal in spleen colonies<sup>10,12</sup>. The number of cells plated in burst assay cultures was low. It thus seems most likely that the distribution observed was due to statistical fluctuations<sup>13</sup> rather than micro-environmental influences<sup>14</sup>. This does not negate the possibility that in complex cell systems, stem cell growth and differentiation can be locally influenced by other cell types in the immediate vicinity. It does, however, suggest that their effects can be studied using soluble factors. The high frequency of CFU-S per burst demonstrated here is an order of magnitude higher than that recently reported for mixed haematopoietic colonies of early fetal liver cell origin<sup>15</sup>. Thus, experiments to analyse the progeny of individual adult marrow stem cells stimulated *in vitro* are now possible. An immediate application will be to study the possible influence of various molecular factors on the self-renewal and differentiative behaviour of these stem cells.

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## Evidence that substance P does not mediate slow synaptic excitation within the myenteric plexus

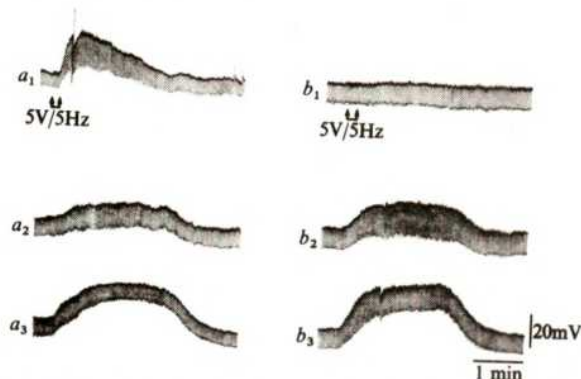
ELECTRICAL stimulation of presynaptic fibres to the so-called AH<sup>1</sup> or type II<sup>2</sup> myenteric neurones in guinea pig small intestine evokes a slow excitatory postsynaptic potential (e.p.s.p.) characterised by long-lasting depolarisation associated with increased membrane resistance and augmented excitability<sup>3</sup>. Two substances have been implicated as possible neurotransmitters for the slow e.p.s.p. Katayama and North reported that application of substance P to myenteric neurones mimicked the slow e.p.s.p.<sup>4</sup>, and J.D.W. and C.J.M. presented several lines of evidence for serotonin as the transmitter substance<sup>5,6</sup>. We now report that methysergide, a drug which abolishes both the slow e.p.s.p. and the action of exogenous serotonin<sup>5,6</sup>, does not affect the action of substance P on guinea pig myenteric neurones. The results suggest that substance P is unlikely to be the neurotransmitter which mediates the slow e.p.s.p.

We used conventional methods, which are described in detail elsewhere, to record intracellular electrical activity and to evoke the slow e.p.s.p. in myenteric ganglion cells of guinea pig small intestine<sup>3</sup>. Substance P from three different sources (Beckman, Serva and Sigma) and methysergide (Sandoz) were applied to the neurones by adding the drugs to the superfusion solution.

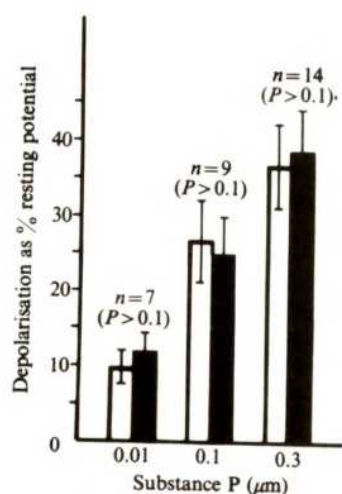
We tested substance P 41 times on 35 ganglion cells from 15 guinea pigs. The compound produced a dose-dependent membrane depolarisation in 30 of the ganglion cells and had no effect on 5 of the cells (Figs 1, 2). In 22 neurones, the depolarisation was accompanied by an increase in membrane resistance, with a decrease in 8. The increase in input resistance did not seem to be related to membrane rectification, and probably reflects decreased membrane conductance for potassium, as suggested by Katayama and North<sup>4</sup>. The membrane depolarisation reached a plateau, and after 45–60 s in substance P, repolarisation, apparently reflecting tachyphylaxis, began. The effects of substance P were reversed when the preparations were washed with drug-free Krebs solution (Fig. 1).

Substance P had the same dose-dependent effects in the presence and absence of methysergide (Fig. 2). On all occasions, the membrane depolarisation produced by the three concentrations of substance P in the presence of methysergide was not significantly different ( $P > 0.10$ ) from the values obtained in its





**Fig. 1** Effects of methysergide on the slow e.p.s.p. and on the action of substance P in a myenteric neurone of guinea pig small intestine. Each neurone was impaled with the microelectrode and the following procedure carried out. (1) Constant current, hyperpolarising pulses were continuously injected into the neurone at 1-s intervals to monitor changes in the neurone's input resistance. (2) Three concentrations of substance P (10, 100 and 300 nmol l<sup>-1</sup>) were applied to the neurone in succession, but in variable order. Each concentration of substance P remained in contact with the neurone for 2 min and was then washed from the superfusion system for a period of 5 min with drug-free Krebs solution before addition of the next concentration of substance P. (3) The slow e.p.s.p. was evoked by electrical stimulation of one of the interganglionic fibre tracts that entered the ganglion. (4) Methysergide 20 μmol l<sup>-1</sup> was then added to the superfusion solution. (5) After 5 min in the presence of methysergide, electrical stimulation was again applied to the fibre tract. The same stimulus parameters were used and the stimulating electrode remained in the same position on the fibre tract throughout the experiment on each neurone. (6) The three concentrations of substance P were then applied in the same manner in the continuous presence of methysergide. (7) The methysergide was washed from the superfusion system and electrical stimulation was again applied to the fibre tract. (8) Current-voltage relationship and rectifying properties of the neuronal membrane were examined. The microelectrode was sometimes dislodged from the cell before completion of the above sequence and this is reflected in the numerical data of Fig. 2. We also sometimes repeated the entire sequence on the same cell. *a*<sub>1</sub>, Slow e.p.s.p. evoked by application of a short train of stimulus pulses (arrows) to one of the fibre tracts that entered the ganglion. Increased amplitude of the electrotonic potentials produced by current injection (increase in baseline width) reflected an increase in the input resistance of the neurone during the depolarising phase of the e.p.s.p. One spike with a long-lasting hyperpolarising after-potential occurred at the peak of the e.p.s.p. *b*<sub>1</sub>, Fibre tract stimulation did not evoke a slow e.p.s.p. in the presence of methysergide. *a*<sub>2</sub> (100 nmol l<sup>-1</sup>), *a*<sub>3</sub> (300 nmol l<sup>-1</sup>). Dose-dependent depolarising action of substance P. *b*<sub>2</sub> (100 nmol l<sup>-1</sup>), *b*<sub>3</sub> (300 nmol l<sup>-1</sup>). The presence of methysergide did not reduce the dose-dependent action of substance P. This neurone in each case was exposed to substance P for 2 min and then substance P washed from the superfusion system.



**Fig. 2** Dose-dependent effect of substance P on the electrical potential across the membrane of guinea pig myenteric neurones in the presence and absence of 20 μmol l<sup>-1</sup> methysergide. The maximal amount of depolarisation produced by each concentration of substance P is expressed as the per cent of the resting potential before application of the peptide. Results given are mean values, s.e.m.s, number of trials per concentration and the level of statistical significance. The arc sine transformation and Student's *t* statistics<sup>8</sup> were used to determine the level of significant difference between the means for each concentration of substance P in the presence and absence of methysergide. □, Substance P; ■, Substance P + methysergide.

absence. Similarly, the changes in input resistance produced by substance P were unaffected by methysergide. On the other hand, on the same cells, the presence of methysergide abolished the stimulus-evoked slow e.p.s.p. (Fig. 1), but this effect was reversed by washing the preparation with drug-free solution. Previous studies indicated that this blocking action of methysergide was due to a specific action at serotonin receptors and not a local anaesthetic action, because electrical stimulation still elicited spike discharge in the neurone when the slow e.p.s.p. was blocked in the presence of methysergide<sup>5,6</sup>.

Our observation that methysergide abolishes the slow e.p.s.p. without affecting the action of substance P makes it unlikely that substance P is the transmitter. Methysergide does block the action of serotonin and this, as well as several other lines of evidence, implicate serotonin as the neurotransmitter<sup>5</sup>. In the brain, the observation that substance P and serotonin occur within the same synaptic vesicles<sup>7</sup> suggests a functional relationship between the two substances; however, such a relationship has not yet been demonstrated.

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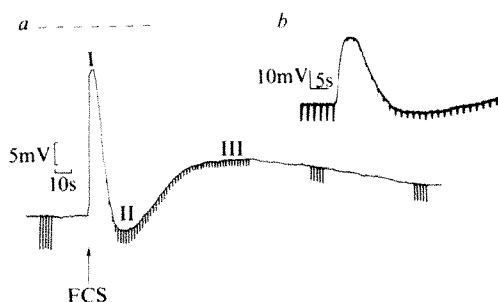
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## Serum triggers a sequence of rapid ionic conductance changes in quiescent neuroblastoma cells

SERUM is required for the growth of nearly all animal cells in culture, but the mechanisms by which serum interacts with cells are largely unknown<sup>1,2</sup>. Evidence exists, however, that the primary site of action of the serum constituents is at the plasma membrane. For example, the first detectable events following serum stimulation of resting fibroblasts involve alterations in membrane transport, such as a stimulation of the (Na<sup>+</sup> + K<sup>+</sup>)ATPase<sup>3,4</sup> and an increase in the uptake of various nutrients<sup>5</sup>. Most of this evidence has been obtained using tracer flux techniques, but the relatively poor time resolution of this method (of the order of minutes) has precluded detection of dynamic membrane changes that may occur within seconds of serum addition. We have applied intracellular electrophysiological techniques in a search for rapid ionic membrane events following serum stimulation of mouse neuroblastoma cells. These cells stop growing (become 'quiescent') after serum removal and begin to extend neurites, but on re-addition of serum the neurites retract and cell division resumes<sup>6,7</sup>. Here we report that the immediate consequence of adding fetal calf serum (FCS) to quiescent neuroblastoma cells is a triphasic



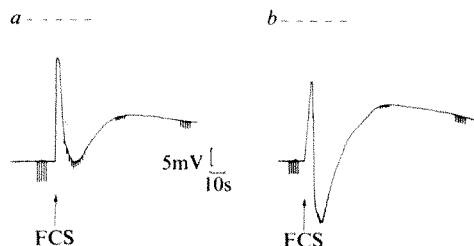


**Fig. 1** *a*, Intracellular recording from a N1E-115 cell during addition of FCS (Flow lot no. 2927028) showing the characteristic triphasic voltage response. Arrow indicates time at which the bathing solution was rapidly exchanged for serum-containing solution (final FCS concentration 30%). Membrane resistance was monitored as the voltage response to brief hyperpolarising current pulses (200 ms; 0.1–0.5 nA). Dashed line represents zero potential level. *b*, Oscilloscope recording of the initial phase, clearly showing the changes in membrane resistance. Membrane resistance during peak of phase I was decreased as much as eight-fold (corrected for voltage dependence). Temperature was  $36 \pm 1^\circ\text{C}$ .

membrane potential response, caused by a series of transient ionic permeability changes, the first of which is a rapid and transient increase in  $\text{Na}^+$  conductance. These conductance changes, which are distinctly different from those underlying electrical excitability, seem to be the first events following serum stimulation and may provide a clue to the mode of action of serum growth factors.

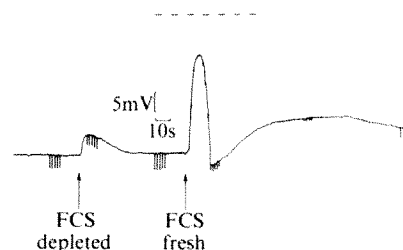
Cells of mouse neuroblastoma clone N1E-115 (ref. 8) were used as the model system because their relatively large cell size and their high specific membrane resistance make them most suitable for electrophysiological study<sup>9,10</sup>. The N1E-115 cells were grown at  $37^\circ\text{C}$  on circular glasses (diameter 1.5 cm; plating density  $2 \times 10^4$  cells per  $\text{cm}^2$ ) for 2 d in Dulbecco's modified Eagle's medium (DMEM) containing 10% FCS and 20 mM HEPES (pH 7.4), and were subsequently incubated in serum-free DMEM for at least 24 h. For experimental convenience dimethyl sulphoxide (DMSO, 2% v/v) was sometimes added to the growth medium to induce an enlargement of the average cell size<sup>11</sup>. DMSO treatment had no effect on the serum-dependent membrane properties.

Intracellular recordings were made with conventional electrophysiological techniques<sup>10</sup>, while the cells were bathed either in serum-free DMEM (0.5 ml;  $35\text{--}37^\circ\text{C}$ ) or in a salt solution with the equivalent ionic composition (130 mM NaCl; 5.5 mM KCl; 1.8 mM  $\text{CaCl}_2$ ; 0.8 mM  $\text{MgCl}_2$ ; 20 mM HEPES, pH 7.4). Heat-inactivated FCS was added by exchanging within a few seconds the serum-free solution for FCS-containing solution.



**Fig. 2** Effects of increased  $\text{Ca}^{2+}$  concentration on the serum-induced voltage response. After FCS addition in normal solution (*a*) the culture was washed and exposed to a 10 mM  $\text{Ca}^{2+}$  saline. The same cell was then re-penetrated and after adjustment of the resting potential to its original level, FCS (containing 10 mM  $\text{Ca}^{2+}$ ) was added again (*b*). Note the marked enhancement of phase II in a hyperpolarising direction, accompanied by a reduction in membrane resistance. Dashed lines represent zero potential level.

N1E-115 cells in serum-free medium maintained resting potentials between  $-30$  and  $-45$  mV and had membrane resistances varying from 10 to 40 M $\Omega$ . As illustrated in Fig. 1, addition of FCS (final concentration 30%) immediately elicited a characteristic triphasic voltage response. The initial phase (I) was a rapid depolarisation, reaching maximum values between  $-5$  and  $-15$  mV within a few seconds, accompanied by a substantial fall in membrane resistance. During the next 5–10 s the membrane repolarised to a potential value near the original resting level (phase II), while the membrane resistance only partially recovered. This hyperpolarising phase was followed by a new depolarising phase (III), accompanied by a gradual decrease in membrane resistance. Phase III reached a plateau value of about  $-25$  mV at 40–70 s following FCS stimulation. Finally, both membrane potential and resistance slowly increased again to new steady-state values which were consistently lower than the prestimulation values. This final recovery usually took 4–8 min, but was occasionally much slower (up to 20 min). Peak values of the serum response were dose dependent, showing saturation at a FCS concentration of about 30%. Addition of DMEM containing a high concentration of bovine serum albumin (BSA, final concentration 25 mg  $\text{ml}^{-1}$ , corresponding to the value in 50% serum) was without any electrical effect. After washing out the FCS-containing medium the serum response could be elicited again in the same cell. This procedure could be repeated several times before the response eventually became 'desensitised'.



**Fig. 3** Effects of growth-depleted and fresh serum (20%) on the same cell. Depleted FCS was obtained by a 3-day exposure of DMEM and 20% FCS to a confluent N1E-115 culture, with daily adjustment of the medium pH to 7.4. Dashed line represents zero potential level.

The ionic mechanisms underlying the sequential phases were evaluated by measuring the null or reversal potential of the distinct peaks as a function of changes in the external ionic concentrations. The null potential of phase I varied between  $-5$  and  $+10$  mV and was dependent on the external  $\text{Na}^+$  concentration. A threefold reduction of  $[\text{Na}^+]$  (choline substituted) resulted in a 20 mV shift of the null potential. A 10-fold reduction of external  $[\text{Ca}^{2+}]$ , however, did not significantly influence the properties of phase I. These results indicate that phase I is mainly caused by an increase in  $\text{Na}^+$  permeability. This phase is not affected by the  $\text{Na}^+$ -channel blocker tetrodotoxin ( $10^{-6}$  g  $\text{ml}^{-1}$ ), however, and corresponds to a peak of inward current in voltage-clamp conditions (not illustrated). Thus, it follows that the serum-activated  $\text{Na}^+$  conductance is clearly different from the voltage-dependent  $\text{Na}^+$  channel underlying the generation of the neuroblastoma action potential<sup>10</sup>.

Phase II reached maximum hyperpolarising values of up to  $-45$  mV. In view of the reduced resistance value this suggests an increase in permeability to  $\text{K}^+$ , which is the only ion with an equilibrium potential ( $E_K$ ) more negative than the resting potential ( $E_K = -75$  mV (ref. 10)). The activation of one component of the  $\text{K}^+$  permeability in neuroblastoma cells has been found to depend on  $\text{Ca}^{2+}$  influx<sup>12</sup>. Enhancing the driving force for  $\text{Ca}^{2+}$  influx by increasing external  $[\text{Ca}^{2+}]$  from 1.8 to 10 mM resulted in a striking shift of the peak value in a hyperpolarising direction towards  $E_K$ , accompanied by a further

decrease of the membrane resistance (Fig. 2). Maximum peak II values in high  $[Ca^{2+}]$  solution approached  $-60$  mV and were reduced by about 40 mV following a 10-fold increase in external  $[K^+]$ .

The dependence of phase II on both external  $Ca^{2+}$  and  $K^+$  strongly suggests that this phase reflects an increase in  $K^+$  permeability, accompanied or preceded by a net  $Ca^{2+}$  influx into the cytoplasm. The resulting increase in intracellular  $[Ca^{2+}]$  is then thought to underlie the  $Ca^{2+}$ -dependent membrane hyperpolarisation<sup>12</sup>.

The reversal potential of phase III was around  $-25$  mV and was sensitive to changes in external  $[Na^+]$  and  $[K^+]$ , whereas changes in external  $[Ca^{2+}]$  were without significant effect. Quantitative evaluation of the ionic selectivity during phase III is difficult, however, as the internal ionic concentrations are likely to change during this prolonged phase of ionic 'leakiness'. We can state, nevertheless, that both  $Na^+$  and  $K^+$  permeability are enhanced during phase III.

The present results are the first direct evidence that serum rapidly triggers dynamic membrane permeability changes to  $Na^+$ ,  $Ca^{2+}$  and  $K^+$  in a specific temporal sequence. In the only previously published electrophysiological study on growth stimulation, serum has been reported to depolarise the membrane of embryonic rat cells, but the underlying ionic mechanisms have not been elucidated<sup>13</sup>. Interestingly, the present data are reminiscent of the first detectable events occurring at egg fertilisation. Sperm-egg interaction triggers a transient 'fertilisation potential', brought about largely by a rapid increase in  $Na^+$  permeability<sup>14</sup>.

A major and intriguing question now is to what extent the rapid serum effects are involved in the triggering of subsequent biochemical events, in particular those which ultimately lead to the initiation of DNA synthesis and cell division. For example, the rapid  $Na^+$  conductance changes might lead to a stimulation of  $Na^+$ -coupled transport processes and to an increase in  $(Na^+ + K^+)ATPase$  activity<sup>15</sup>. Figure 3 shows that application of growth-depleted FCS, incapable of supporting growth, fails to induce a significant electrophysiological response. This result supports the view that the rapid ionic events are due to the growth-promoting factors in serum. Future investigations of early changes in membrane-linked events as well as characterisation of the serum factors involved should clarify the possible role that the phenomena described here have in growth control.

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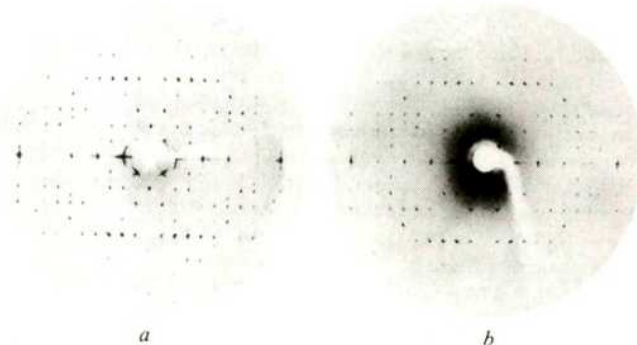
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## Gramicidin A crystals contain two cation binding sites per channel

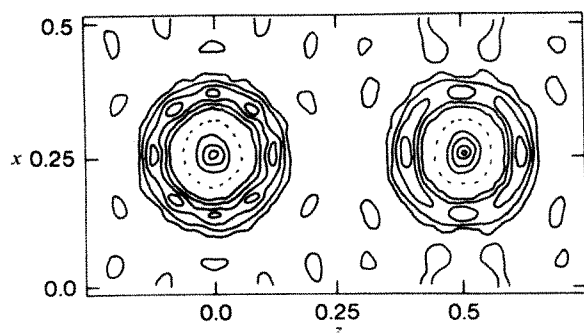
GRAMICIDIN A, a pentadecapeptide antibiotic from *Bacillus brevis*, dimerises to form transmembrane channels in biological and synthetic membranes<sup>1-4</sup>. These channels are permeable to alkali cations and protons, impermeable to anions and blocked by calcium ions<sup>5-8</sup>. The high transport rate ( $\sim 10^7$  ions  $s^{-1}$ ), moderate selectivity and simplicity of the gramicidin channel make it an attractive model for elucidating the mechanism of ion transport in biological membranes. Our previous X-ray crystallographic study showed that crystals of native gramicidin and of its complex with caesium ion contain cylindrical channels which are formed from helical dimers of gramicidin<sup>9</sup>. We also found that the binding of caesium leads to a large conformational change: the length of the channel decreases from 32 to 26 Å, whereas the diameter of the cylinder formed by the peptide main chain atoms increases from about 5 to 6.8 Å. We report here X-ray studies showing that the same conformational change is induced by the binding of the potassium ion. Furthermore, the isomorphism of the  $K^+$ -complex and  $Cs^+$ -complex crystals enabled us to calculate difference Fourier projections and define the location of the ion-binding sites.

Crystals of the  $K^+$ -complex of gramicidin were grown at room temperature over a 1-yr period from a solution of 21 mM KSCN and 21 mM gramicidin (commercial mixture of gramicidins A, B and C from ICN Life Sciences Group) in methanol. X-ray diffraction data to 2.5 Å resolution were collected as described previously<sup>9</sup>. The unit cell dimensions of the  $K^+$ -gramicidin crystal are virtually the same as those of  $Cs^+$ -gramicidin (Table 1). Many intensity changes due to the substitution of  $K^+$  for  $Cs^+$ , a difference of 36 electrons, are evident in the  $0kl$  precession photograph shown in Fig. 1. A three-dimensional difference Patterson map was calculated using  $(|F_{Cs}| - |F_K|)^2$  as coefficients. The Harker peaks showed that the space group is  $P2_12_12$ , resolving an earlier ambiguity<sup>9</sup>. The 12 highest peaks in the difference map at 5 Å resolution were interpreted in terms of Harker peaks and cross peaks arising from four cation-binding sites in the asymmetric unit. The positions of anions could not be determined because the thiocyanate counterion was the same in both crystals. The cation-binding sites were refined individually, then in pairs, and finally all together at 5 Å and at 3 Å resolution, using the three centrosymmetric projections by the method of Dickerson *et al.*<sup>10,11</sup>. When the occupancies and temperature factors of the four sites were constrained to be equal, the centric  $R$  factor<sup>12</sup> was 0.36 at 3.0 Å resolution, and the Kraut  $R$  factor<sup>13</sup> was 0.088. The centric  $R$  factor dropped to 0.34 when the individual occupancies and temperature factors were allowed to vary. Table 2 gives the coordinates of these sites. Phases of the



**Fig. 1**  $(0kl)$  Precession photographs of crystals of  $K^+$ -gramicidin (a) and  $Cs^+$ -gramicidin (b). The average difference in structure factor between these isomorphous crystals is 16%. A number of significant intensity changes are evident by visual comparison of the two patterns.





**Fig. 2** Centric  $y$ -axis projection Fourier map of  $K^+$ -gramicidin at 2.9 Å resolution.

centric reflections were then calculated by the single isomorphous replacement method. Projection difference Fourier maps showed no additional sites. There remains an ambiguity in the phase of each general reflection when only a single isomorphous heavy atom derivative is available. As the heavy atom array is nearly centrosymmetric, a three-dimensional Fourier map calculated from this single derivative is also nearly centric and hence not interpretable at the atomic level.

A projection Fourier map of the  $K^+$  complex is shown in Fig. 2. The asymmetric unit contains two cylindrical channels. Each channel consists of two polypeptide chains and contains two bound cations. The central density in each channel corresponds to the bound ions. The channels at  $z = 0$  and  $z = 0.5$  are not crystallographically equivalent but appear very similar in projection (Fig. 2). They give rise to a pseudo-cubic-centred and pseudo-tetragonal packing<sup>9</sup>, and so their structures must be nearly the same. The axes of the channels are coincident with crystallographic  $2_1$  axes, thus limiting the maximum length of a channel to 26 Å, half of the 52 Å unit cell length. The pair of ion sites at  $z = 0$  and  $z = 0.5$  are related by a translation of nearly 0.25 in  $y$ , supporting the notion that the crystallographically independent channels have very similar structures.

**Table 1** Unit cell parameters of crystals of  $K^+$ - and  $Cs^+$ -gramicidin

Ion	Space group	$a$	$b^*$	$c$
$K^+$	$P2_12_12$	32.11 Å	52.23	31.09
$Cs^+$	$P2_12_12$	32.07 Å	52.29	31.20

\* Channel axis.

Conductance and spectroscopic studies of analogues of gramicidin A indicate that the two strands in the dimeric channel are antiparallel<sup>14-16</sup>. Hence, the two cation-binding sites in a channel are probably equivalent. The two possible symmetric arrangements of these sites are depicted in Fig. 3, which shows that they are either 2.5 Å from the ends of the channel or, alternatively, 2.5 Å on each side of the centre of the channel. Our finding of two cation-binding sites per channel in the crystalline state agrees with  $^{86}Rb^+$  flux studies<sup>17</sup> showing the bi-directional fluxes in the gramicidin channel in bilayer membranes are not independent and that two sites are simultaneously occupied. Moreover, the stoichiometry of  $K^+$  binding to gramicidin in methylene chloride is one per polypeptide chain<sup>18</sup>, and so two sites per dimeric channel are expected. The dependence of the conductance on the cation concentration also shows that at least two cations can simultaneously interact with the gramicidin channel<sup>19,20</sup>.

The binding of  $K^+$ , like that of  $Cs^+$ , shortens the channel from 32 to 26 Å and widens its diameter from 5 to 6.8 Å. The diameter cited here refers to the approximate average distance between the centres of the peptide backbone atoms on either side of the channel. The space available for ion binding is about 3.0 Å smaller (the sum of the van der Waals radii of the atoms on either side of the channel). It is interesting that the conformational changes elicited by the binding of these ions are

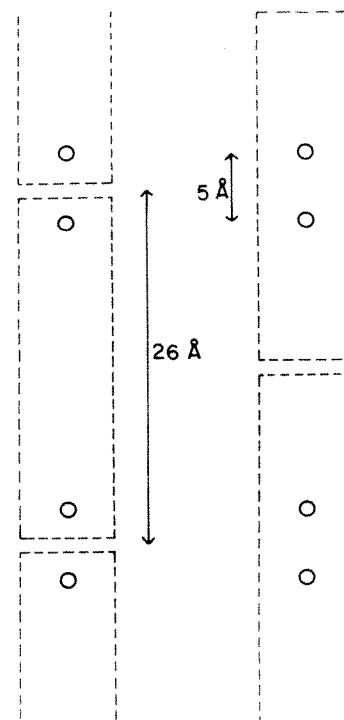
**Table 2** Cation-binding sites in an asymmetric unit in crystals of  $K^+$ - and  $Cs^+$ -gramicidin

Site	$x$	$y^*$	$z$
1	8.1 Å	-1.1 Å	-0.2 Å
2	8.0	4.2	0.3
3	7.9	11.6	16.5
4	8.5	16.9	16.1

The unit cell contains a total of 16 cation-binding sites. Two of the four sites in an asymmetric unit are situated near ( $x = 0.25$ ;  $z = 0$ ) and separated by 5 Å along the channel axis  $y$ , and another two sites are located near ( $x = 0.25$ ;  $z = 0.5$ ) and separated by 5 Å in  $y$ . Each of these four sites gives rise to three additional ones by space group symmetry. In particular, each site is repeated by crystallographic symmetry at the same  $x$  and  $z$  coordinates and translated by  $y = 0.5$  (26 Å). Hence, each site is separated from its neighbours along the  $y$ -axis by 5 Å on one side and by 21 Å on the other side.

virtually identical although their ionic radii differ significantly (1.33 Å for  $K^+$ , 1.67 Å for  $Cs^+$ , ref. 21). We have also found that a new tetragonal form of a  $CsSCN$  complex of gramicidin is isomorphous with a thallos acetate complex.

Our results thus far are consistent with the existence of two major conformations for the gramicidin channel, an ion-free conformation observed in two crystal forms<sup>9</sup>, and an ion-bound conformation observed in the crystal complexes with  $Cs^+$ ,  $K^+$  and  $Tl^+$ . The ion-free conformation seems to be substantially independent of whether the solvent is methanol, ethanol or water. The helical Patterson peaks are the same for the methanol and ethanol crystals<sup>9</sup>. Furthermore, ethanol can be exchanged for 50% ethanol-50%  $H_2O$  with almost no change in the diffraction pattern. The large structural difference between forms with and without ions is likely to be due to a rearrangement in the hydrogen-bonding pattern, similar to that observed in valinomycin, an ion-carrier<sup>22,23</sup>. Some of the peptide  $C=O \cdots H-N$  hydrogen bonds are probably broken as the peptide carbonyl oxygens tilt inwards to coordinate the incoming cation. These interactions could not occur in the 5 Å diameter of the native channel because of steric hindrance. It seems likely that the gramicidin channel widens on binding



**Fig. 3** Schematic drawing showing the two possible symmetric arrangements of the cation-binding sites in a gramicidin channel. Adjacent sites are separated by 5 Å and by 21 Å in a 26 Å-long channel.



cations to permit peptide carbonyl-metal coordination, which compensates for some of the free energy lost in partially dehydrating the transported ion.

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## Seminalplasmin—an antimicrobial protein from bovine seminal plasma which acts in *E. coli* by specific inhibition of rRNA synthesis

SEMINAL PLASMA—the fluid in which spermatozoa are ejaculated—has been known to inhibit reversibly both RNA and protein synthesis in sperm cells<sup>1-3</sup>. Here we report the isolation from seminal plasma, purification to homogeneity, and characterisation of a protein which we have called 'seminalplasmin', that specifically inhibits rRNA synthesis in *Escherichia coli* and is highly antimicrobial.

As transcription and translation in bovine spermatozoa are exclusively mitochondrial<sup>4,5</sup> and as there are close similarities between mitochondrial and prokaryotic transcription and translation, we used inhibition of the growth of *E. coli* to assay seminalplasmin during purification. Dialysed bovine seminal plasma was passed through a DEAE-Sephadex column and seminalplasmin was purified to homogeneity from the unadsorbed fraction by successive fractionation on a CM-Sephadex, a Sephadex G-75 and a [5'-(*p*-aminophenylphosphoryl)uridine-2'(3')-phosphate]-agarose (APU-agarose) affinity column (see Fig. 1). The use of the latter column removed traces of RNase SPL<sup>6</sup>. The DEAE-Sephadex column removed an inhibitor, 'antiseminalplasmin', which we have partially purified. Seminalplasmin obtained after the affinity chromatography was

**Table 1** Effect of seminalplasmin at various stages of purification, on the growth of *E. coli*

Stage of purification	Concentration of protein ( $\mu\text{g ml}^{-1}$ )	Inhibition of the growth of <i>E. coli</i> (%)
CM-Sephadex peak 4	40	100
	20	100
	10	40
	5	0
Sephadex G-75 fraction C	40	100
	20	100
	10	90
	5	50
Homogeneous seminalplasmin (after affinity chromatography)	40	100
	20	100
	10	90
	5	50

Aliquots of a logarithmically growing culture of *E. coli* W 160-37 ( $A_{760}$ , 0.01) were incubated with various concentrations of seminalplasmin-containing protein fraction. After 6 h, the  $A_{760}$  of the culture was measured. The absorbance of the culture to which no seminalplasmin was added,  $A_{760}$  of 0.5-0.6, was taken as 100 for the purpose of calculation of the percentage inhibition. For details of the fractions used for the growth-inhibition assay, see Fig. 1 and text. Affinity chromatography removed traces of RNase SPL contamination in Sephadex G-75 fraction C which was otherwise pure seminalplasmin. An  $A_{280}$  of 1 was taken to be equivalent to 1 mg of protein throughout this study. The composition of the growth medium was as follows ( $\text{g l}^{-1}$ ):  $\text{NH}_4\text{Cl}$  0.5;  $(\text{NH}_4)_2\text{SO}_4$  0.5;  $\text{KH}_2\text{PO}_4$  13.6;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.02;  $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$  0.0156; glucose 20; L-arginine hydrochloride 0.1. The above assay for growth inhibition would not detect the presence of a small number (<2% of the control) of viable organisms in the culture. Similar results were obtained with the other strains of *E. coli* tried.

homogeneous in both non-SDS and SDS polyacrylamide gel electrophoresis (PAGE)<sup>7-9</sup> (Fig. 1), in an analytical ultracentrifuge (sedimentation coefficient, 1.04S), and in an isoelectric focusing run on a column<sup>10</sup> (isoelectric point, 9.8); it was free of any detectable RNase, DNase or protease activity<sup>11</sup>.

The amino acid composition of seminalplasmin is, in mol per 100 mol of total amino acids (except Trp) was: Lys 12.30; Arg 9.60; His 4.15; Asx 11.26; Glx 4.92; Phe 3.95; Tyr 1.66; Pro 3.13; Cys 2.98; Thr 1.75; Ser 7.20; Gly 5.75; Ala 7.90; Val 0.63; Leu 14.20; Ile 1.80. The protein is rich in basic amino acids and contains no methionine.

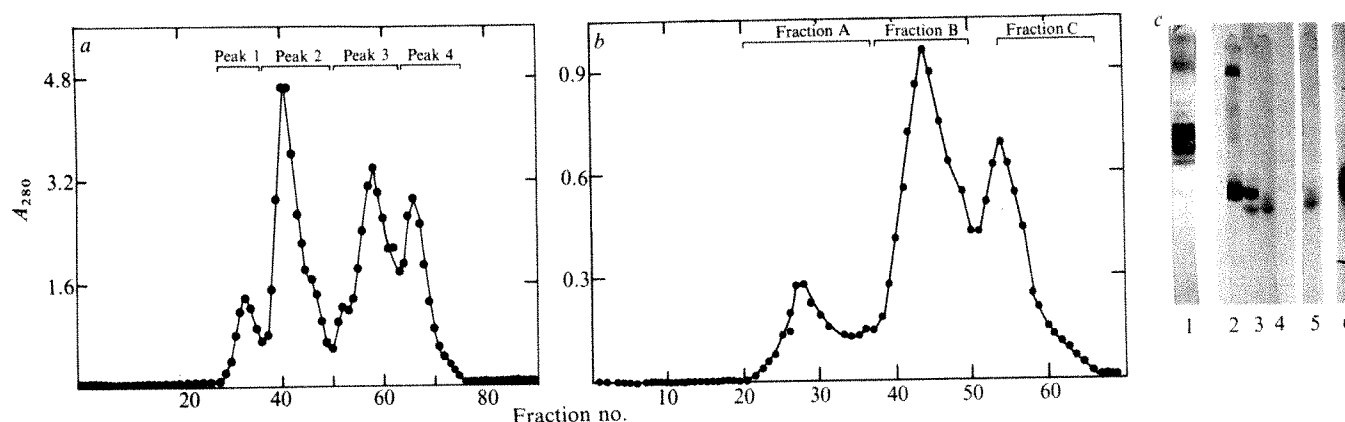
The molecular weight of seminalplasmin was 17,000 by SDS-PAGE carried out as in ref. 8; 10,600 by SDS-PAGE carried out according to Laemmli<sup>9</sup>; 8,000 by ultracentrifugation, and 19,800 (minimum value) when calculated from the amino acid composition. The discrepancy between these values may be a reflection of certain uncommon structural features—such as an abnormally high axial ratio—in the protein.

The average yield of pure seminalplasmin was 18 mg per 100 ml of semen (average ejaculate, 4 ml); the total content of seminalplasmin in bovine semen is probably twice as much.

**Table 2** Effect of seminalplasmin on the growth of microorganisms

Petri dish no.	Microorganism	Description	Test substance Amount per disk (nmol)	Diameter of the zone of inhibition (mm)
1	<i>Candida albicans</i>	Seminalplasmin	5	15
		Streptomycin	17	0
		Chloramphenicol	53	0
2	<i>Salmonella typhimurium</i>	Seminalplasmin	5	15
		Streptomycin	17	8
		Chloramphenicol	53	15
3	<i>E. coli</i>	Seminalplasmin	5	15
		Streptomycin	17	8
		Chloramphenicol	53	15

Exponentially growing broth cultures were transferred to medium 199-Earle's salts plates (one for each organism) using a heavy inoculum which, on normal growth, would give a 'mat'. Disks impregnated with the stated amount of substance were placed on the surface of the culture just after the inoculation, and the diameter of the clear zone measured after 20 h at 37 °C. The strains used were isolated at the Institute of Preventive medicine, Hyderabad, where the testing was done. The two bacterial strains were partially resistant to streptomycin.



**Fig. 1** Purification of seminalplasmin. Seminal plasma (200 ml) obtained from bovine semen following removal of spermatozoa by centrifugation was dialysed against Tris-HCl buffer (0.006 M, pH 7.4) and the dialysed plasma passed through a DEAE-Sephadex column (5 × 60 cm) equilibrated with the above buffer. The fraction that came out unadsorbed from the DEAE-Sephadex column was chromatographed on a CM-Sephadex C-50 column (2 × 45 cm) equilibrated with the Tris-HCl buffer and eluted with a linear gradient of 0–0.8 M NaCl in the same buffer. The peaks were pooled as marked, dialysed against water and lyophilised to give the crude seminalplasmin. In some runs, peaks 3 and 4 were not separated. Peaks 1 and 2 had no detectable seminalplasmin activity, while peak 3 had some activity which was possibly due to cross contamination with peak 4. The crude seminalplasmin (peak 4 of a, 100 mg; where peaks 3 and 4 were not separated, the trailing part of the combined peak was used) was then chromatographed on Sephadex G-75 (2.6 × 170 cm) equilibrated with Tris-HCl buffer (pH 7.4) and eluted with the same buffer. The pooled fractions A, B and C were dialysed separately against water and lyophilised. Fraction C was dissolved in 0.02 M acetate buffer (pH 5) and chromatographed on a APU-agarose affinity column (0.8 × 6 cm) equilibrated with the acetate buffer. The column was washed with the same buffer; the unadsorbed fraction consisted of pure seminalplasmin (the adsorbed fraction contained RNase SPL). a and b, typical CM-Sephadex and Sephadex G-75 column runs, respectively. c, Polyacrylamide gel electrophoresis runs of various fractions. (1)–(5), Non-SDS PAGE<sup>7</sup>; (6) SDS-PAGE<sup>8</sup>. (1) CM-Sephadex peak 4; (2) Sephadex fraction A; (3) Sephadex fraction B; (4) Sephadex fraction C; (5) and (6) the part of Sephadex fraction C that was unadsorbed on the APU-agarose affinity column (pure seminalplasmin). (1) Expt 1; (2) to (5) expt 2; (6) expt 3. Each gel was loaded with 100 µg of protein. The gels were stained with Coomassie blue (0.1%) in ethanol:water:acetic acid (45:45:10 v/v). Several protein fractions which could be separated on non-SDS PAGE in (1) moved together on SDS-PAGE done as in (6).

Seminalplasmin potently inhibited the growth of Gram-positive and Gram-negative bacteria and yeasts. In liquid cultures grown in a synthetic medium, starting from an inoculum of  $10^6$ – $10^7$  colony forming units (CFU)  $\text{ml}^{-1}$  for bacteria and  $10^5$ – $10^6$  CFU  $\text{ml}^{-1}$  for yeasts, seminalplasmin completely inhibited the growth of *Streptococcus faecalis*, *E. coli* and *Cryptococcus neoformans* at 50–100 µg (2.5–5.0 nmol), 25–50 µg (1.25–2.50 nmol), and 12.5–25 µg (0.63–1.26 nmol) per ml, respectively (see Table 1). Comparable inhibition of growth of organisms considered sensitive to established antimicrobial agents was (nmol  $\text{ml}^{-1}$ ): penicillin and streptomycin, 1.7–8.5; chloramphenicol, 9–27; tetracyclines, 2.2–11; erythromycin, 1.4–2.8; bacitracin, 4.5–9; polymyxin, 0.9–2.2. In disk agar assays, seminalplasmin was active against *Salmonella typhimurium* and *Candida albicans*; it was also active against *Bacillus subtilis* and *Staphylococci* in another type of assay. Seminalplasmin, however, showed no activity against *Pseudomonas* and *Proteus* spp. Results of some typical antimicrobial assays of crude seminalplasmin fractions and the pure protein are shown in Tables 1 and 2.

Seminalplasmin is bacteriocidal and not bacteriostatic. When a culture of *E. coli* W 160-37 containing  $\sim 10^7$  cells  $\text{ml}^{-1}$  was incubated with seminalplasmin ( $\geq 40$  µg  $\text{ml}^{-1}$ ) for 7 h at 37 °C (when the control contained  $2.3 \times 10^8$  cells  $\text{ml}^{-1}$ ), and then 50 µl of the culture plated on nutrient agar, complete inhibition of growth was normally obtained. Occasional colonies of apparently seminalplasmin-resistant cells were seen.

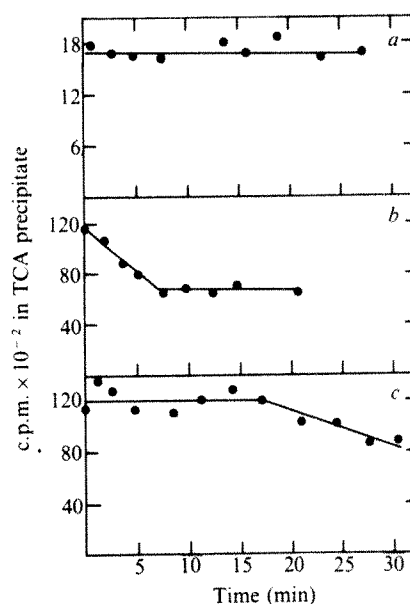
The concentration of seminalplasmin needed to inhibit the growth of *E. coli*, as assayed in Table 1, depended on the number of cells present in the culture; whereas for  $10^7$  cells  $\text{ml}^{-1}$ , the concentration required was 10–20 µg  $\text{ml}^{-1}$ , for  $5 \times 10^7$  cells  $\text{ml}^{-1}$ , 40–50 µg  $\text{ml}^{-1}$ , for  $10^8$  cells  $\text{ml}^{-1}$ , 60–70 µg  $\text{ml}^{-1}$ , and for  $2 \times 10^8$  cells  $\text{ml}^{-1}$ , 100–110 µg  $\text{ml}^{-1}$  of seminalplasmin was required.

Seminalplasmin is relatively heat stable; when *E. coli* cells ( $10^7$   $\text{ml}^{-1}$ ) were grown in the presence of seminalplasmin (40 µg  $\text{ml}^{-1}$ ) heated at 90 °C for 10 min, over 95% inhibition of growth was obtained at 6 h, compared with 100% inhibition with untreated seminalplasmin. Lyophilised, pure seminalplasmin is stable for at least 2 yr at 4 °C; in aqueous solution it is stable for at least 6 months when stored frozen.

A 52% inhibition of the synthesis<sup>3,4</sup> of RNA in bovine spermatozoa, measured over a period of 2.5 h, was seen with seminalplasmin (1 mg  $\text{ml}^{-1}$ ) preincubated with the cells for

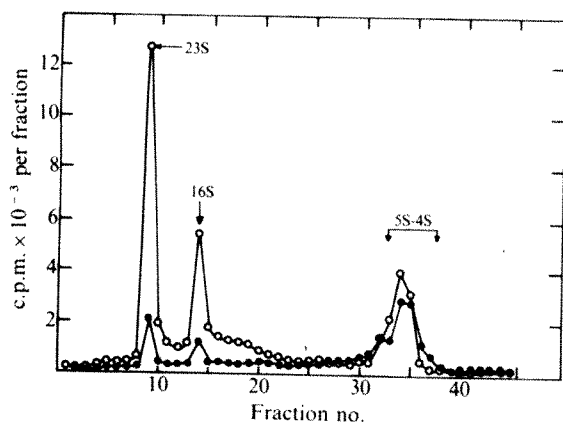
30 min, suggesting that at least part of the total transcription-inhibiting activity of bovine seminal plasma is due to seminalplasmin. Seminalplasmin had no effect on respiration in spermatozoa studied over a period of 2 h using fructose (the semen sugar) as the oxidisable substrate.

Seminalplasmin (100 µg  $\text{ml}^{-1}$ ) had no effect on the rates of synthesis of DNA and protein in *E. coli* W160-37—at least up to 15 min—in pulse-labelling experiments (Fig. 2a, c). The rate of incorporation of <sup>3</sup>H-uridine into RNA, however, progressively fell to about 50% of the control at 8 min (Fig. 2b); beyond this



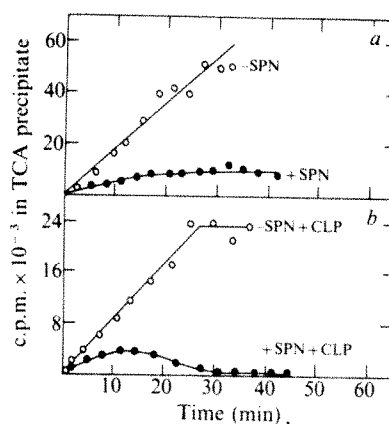
**Fig. 2** Effect of seminalplasmin on the rate of synthesis of DNA (a), RNA (b) and protein (c). a, A sample of 6 ml of a logarithmically growing culture of *E. coli* ( $A_{760}$ , 0.3) was incubated with seminalplasmin (100 µg  $\text{ml}^{-1}$ ). At each time point, a 0.5-ml aliquot of the culture was pulse-labelled for 20 s with 13.3 µCi (0.41 µg) of <sup>3</sup>H-thymidine added in 0.2 ml of the growth medium. The reaction was stopped with 5 ml of cold 5% trichloroacetic acid (TCA), and 0.1 ml of 0.5% bovine serum albumin was added. The precipitate was filtered on a Whatman 3 MM filter, washed three times with 10 ml of cold 5% TCA, dried and counted. b, The experimental protocol was as in a but pulse-labelling was with <sup>3</sup>H-uridine (13.3 µCi, 0.32 µg). c, Pulse labelling was as in a but with 1 µCi (0.65 µg) of <sup>14</sup>C-leucine. The TCA precipitate was heated with 5% TCA at 95 °C for 30 min and then filtered, washed and counted. Counting efficiency was 40% for <sup>3</sup>H and 80% for <sup>14</sup>C.

time no further decrease in rate of RNA synthesis was observed, suggesting that seminalplasmin may be specifically inhibiting the synthesis of a certain type(s) of RNA. In pulse-labelling experiments such as those shown in Fig. 2b, the label is known to be roughly equally distributed between mRNA and rRNA. Since protein synthesis was not affected for at least 20 min, it seemed unlikely that seminalplasmin was inhibiting the synthesis of mRNA. In *E. coli* cells ( $A_{760}$ , 0.2) preincubated with seminalplasmin ( $170 \mu\text{g ml}^{-1}$ ) for 15 min, the induction of  $\beta$ -galactosidase with isopropylthiogalactoside was also normal. We showed that seminalplasmin specifically inhibits the synthesis of rRNA in *E. coli*, by analysing on PAGE the RNA labelled in 20 min in the presence of seminalplasmin ( $100 \mu\text{g ml}^{-1}$ ) in a growing culture of *E. coli* which had been preincubated with the protein for 15 min (Fig. 3). Seminalplasmin inhibited the labelling of rRNA by 85%; tRNA synthesis was inhibited by  $\sim 10\%$ , probably through inhibition of the transcription of genes for tRNA that are a part of the rRNA cistrons in *E. coli*<sup>12</sup>. In line with these observations, addition of seminalplasmin ( $100 \mu\text{g ml}^{-1}$ ) to *E. coli* cultured in the presence of  $^3\text{H}$ -uridine almost completely stopped the increase in the label appearing in total RNA after 12 min in cells growing in the absence of chloramphenicol (Fig. 4a), and completely stopped it in chloramphenicol-treated non-growing cells (Fig. 4b) in which the RNA that is labelled is almost entirely transcribed from rRNA cistrons. In cells treated with chloramphenicol and seminalplasmin, the RNA labelled in the first 12 min (the time presumably required for building up of the intracellular concentration of seminalplasmin necessary for its full inhibitory effect) was subsequently degraded (Fig. 4b); this degradation—also observed in the presence of rifampicin instead of seminalplasmin<sup>13</sup>—may be related to the instability of rRNA synthesised in the presence of chloramphenicol, hence not protected by ribosomal proteins.



**Fig. 3** Fractionation by polyacrylamide gel electrophoresis of *E. coli* RNA synthesised in the presence or in the absence of seminalplasmin. Aliquots of 5 ml of a logarithmically growing culture of *E. coli* ( $A_{760}$ , 0.30) were incubated with or without seminalplasmin ( $200 \mu\text{g ml}^{-1}$ ). At 10 min,  $^3\text{H}$ -uridine ( $45 \mu\text{Ci}$ ,  $10.1 \mu\text{g}$ ) was added to both the aliquots. At 35 min,  $50 \mu\text{l}$  of 2% sodium azide and 1 mg of  $^{12}\text{C}$ -uridine were added and the solution was immediately kept in an ice-salt bath. Unlabelled *E. coli* RNA (2 mg) was then added and RNA isolated from both the samples by extraction with aqueous phenol at pH 5.2. RNA solutions were dialysed and fractionated on a 2.5% polyacrylamide gel, and the gels cut and counted. Arrows indicate the positions of the carrier (non-radioactive) 23S, 16S and 5S-4S RNA fractions visualised by staining with methylene blue (0.01% in 0.01 M acetate buffer, pH 4.7).  $\circ$ , Without seminalplasmin;  $\bullet$ , with seminalplasmin.

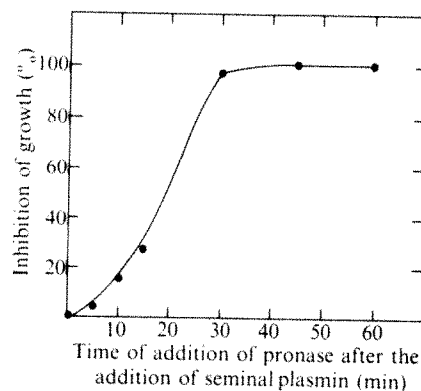
In *E. coli* in the presence of a high concentration of seminalplasmin ( $200 \mu\text{g ml}^{-1}$ ), the rate of protein synthesis also started to decrease after 10 min; the fall was linear, reaching 50% of control at 26 min. In the presence of  $100 \mu\text{g}$  of seminalplasmin per ml, the rate of protein synthesis started to fall (linearly) only after 15–20 min, reaching 50% of the control after 40 min. An eventual decline in the rate of protein synthesis is to be expected as a consequence of cessation of rRNA synthesis.



**Fig. 4** a, Effect of seminalplasmin on RNA synthesis in *E. coli*. Aliquots of 11 ml of a logarithmically growing culture of *E. coli* ( $A_{760}$ , 0.3) were incubated for various periods at  $37^\circ\text{C}$  with (or without) seminalplasmin ( $100 \mu\text{g ml}^{-1}$ ), in the presence of  $^3\text{H}$ -uridine ( $3.9 \mu\text{Ci ml}^{-1}$ ,  $7.88 \mu\text{g ml}^{-1}$ ). At each time point, a 0.5-ml aliquot from each of the incubated cultures was precipitated with 5 ml of ice-cold 5% TCA containing an excess of  $^{12}\text{C}$ -uridine; the radioactivity ( $x$ ) of the precipitate was taken as a measure of labelling of the total nucleic acid. Another 0.5-ml aliquot from the culture was treated with an equal volume of 1 M KOH for 18 h at  $37^\circ\text{C}$ , neutralised and then made 5% with respect to TCA; the radioactivity ( $y$ ) in the precipitate gave the extent of labelling of DNA.  $x - y$  gave the radioactivity in RNA. b, Effect of seminalplasmin on RNA synthesis in a chloramphenicol-inhibited culture of *E. coli*. Aliquots of 11 ml of a logarithmically growing culture of *E. coli* ( $A_{760}$ , 0.26) were incubated at  $37^\circ\text{C}$  with (or without) seminalplasmin ( $212 \mu\text{g ml}^{-1}$ ), in the presence of chloramphenicol ( $149 \mu\text{g ml}^{-1}$ ) and  $^3\text{H}$ -uridine ( $1.86 \mu\text{Ci ml}^{-1}$ ,  $7.48 \mu\text{g ml}^{-1}$ ). Aliquots were taken from each culture at various times and the radioactivity in RNA and DNA determined as in (a). SPN, seminalplasmin; CLP, chloramphenicol.

The antimicrobial and *in vivo* rRNA-transcription inhibiting activities of seminalplasmin were completely abolished by pronase, trypsin, chymotrypsin or papain.

Figure 5 shows the effects of adding pronase ( $30 \mu\text{g ml}^{-1}$ ) to cultures of *E. coli* ( $A_{760}$  0.01) exposed to seminalplasmin ( $50 \mu\text{g ml}^{-1}$ ) for various intervals between 0 and 60 min. Addition of pronase at 0 min showed no inhibition of growth. Increased inhibition of growth was obtained as the time of exposure to seminalplasmin in the absence of pronase increased. A 30-min exposure to seminalplasmin ( $50 \mu\text{g ml}^{-1}$ ) seemed to be sufficient to allow the *E. coli* cells at  $\sim 10^7$  cells  $\text{ml}^{-1}$  to take up enough seminalplasmin to inhibit the growth completely. The experiments described in Figs 2 and 4 suggest that, at a higher concentration ( $100 \mu\text{g ml}^{-1}$ ), enough seminalplasmin enters the cells ( $\sim 3 \times 10^8 \text{ ml}^{-1}$ ) in 8–12 min to exert its maximal inhibitory effect on rRNA synthesis.



**Fig. 5** Effect of pronase on the growth of *E. coli* preincubated with seminalplasmin for various periods. To a set of tubes (one for each time point) containing the growth medium (5 ml) and seminalplasmin ( $50 \mu\text{g ml}^{-1}$ ), was added 1 ml of a logarithmically growing culture of *E. coli* ( $A_{760}$ , 0.06). The tubes were incubated at  $37^\circ\text{C}$  and, at each time point, pronase ( $30 \mu\text{g ml}^{-1}$ ) was added to one tube. After 6 h,  $A_{760}$  of all the cultures was measured. For calculation of the percentage inhibition of growth in the seminalplasmin and pronase-treated cultures, the growth ( $A_{760}$ , 0.5–0.6) obtained in a control culture to which no seminalplasmin but pronase was added, was taken as 100. Pronase, at the concentrations used, had no effect on the growth of *E. coli*; at higher concentrations ( $100 \mu\text{g ml}^{-1}$ ) it stimulated growth.



Seminalplasmin is apparently the fourth antimicrobial protein to be isolated from mammalian sources—the others being lysozyme,  $\beta$ -lysin, and a recently described protein from polymorphonuclear leukocytes<sup>14</sup>. After colicin E<sub>3</sub>, it appears to be the first protein for which there is strong evidence that it enters an intact bacterial cell. As seminalplasmin acts on a variety of microorganisms, it is unlikely that its entry into the cell—unlike that of colicin E<sub>3</sub>—is receptor-mediated. Seminalplasmin is also the first inhibitor shown to specifically inhibit the transcription of rRNA in a whole cell.

We first considered the possibility that seminalplasmin may have a role in fertilisation. In a year-long experiment we looked for a correlation between the net antibacterial activity (a function of the ratio of seminalplasmin to antiseminalplasmin, and the amount of each, in seminal plasma) of over 1,000 samples of bovine seminalplasma derived from 126 animals belonging to eight different breeds of two different bovine species (buffalo-bull and cow-bull), and the fertility of semen samples from which the seminal plasma was obtained (P.M.B. *et al.*, unpublished). The fertility was determined by artificial insemination of nearly 2,200 cows and buffaloes. To allow for seasonal variations, the samples were collected over the entire calendar year. Computer analysis of the data has shown no simple correlation between the net antibacterial activity of seminal plasma and the fertilising ability of the semen sample from which the particular batch of seminal plasma was derived.

It is tempting to speculate that the function of seminalplasmin is to act as an antimicrobial agent. It could provide protection either to the male or to the female through deposition of seminal plasma in the reproductive tract during sexual intercourse, or to both. If the natural function of seminalplasmin were to provide protection to the female, one might expect to find a higher incidence of microbial infection of the female genital tract in women who are celibate or have sexual intercourse only rarely, than in women who have frequent sexual intercourse. We are investigating this possibility.

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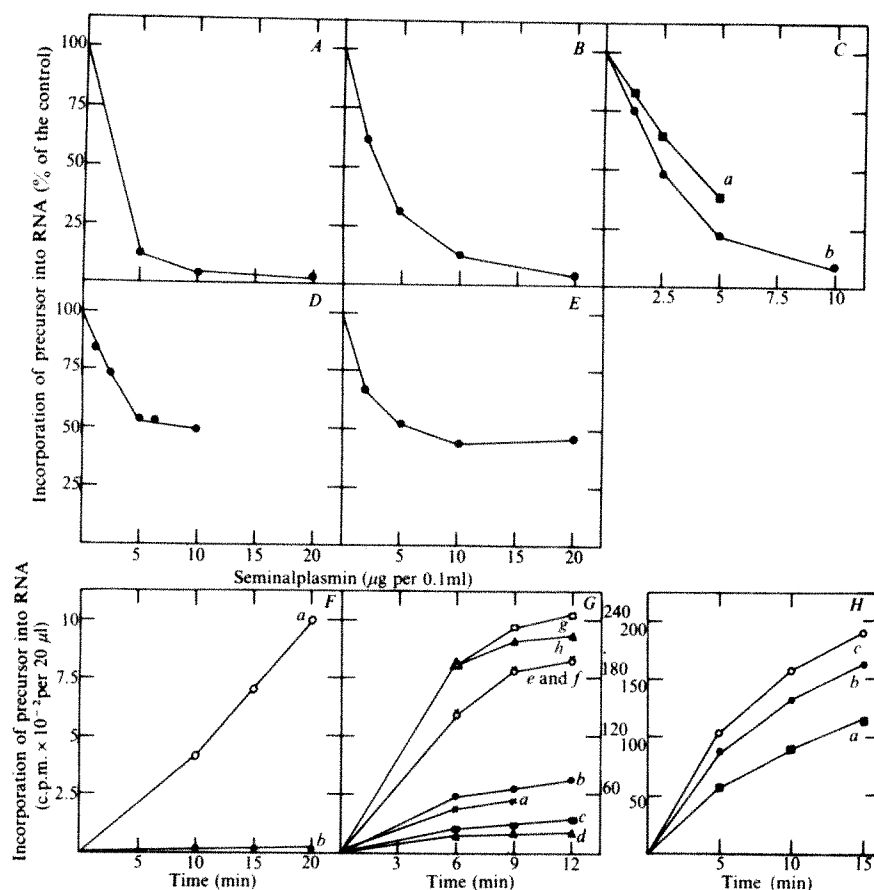
## Seminalplasmin is a potent inhibitor of *E. coli* RNA polymerase *in vitro*

SEMINALPLASMIN, a protein isolated from bovine seminal plasma, specifically and almost completely inhibits synthesis of rRNA in whole *Escherichia coli* cells at extracellular concentrations lower than those needed for bacteriocidal action of many established antibacterial agents<sup>1</sup>. We show here that seminalplasmin also strongly inhibits the transcription of various natural and synthetic templates by *E. coli* RNA polymerase *in vitro*, and that it does so by binding strongly to the polymerase. Seminalplasmin is, to our knowledge, the first protein isolated from an eukaryote to inhibit RNA polymerase. There is, however, a protein synthesised in *E. coli* following a phage infection and coded by the phage genome, which appears to inhibit *E. coli* RNA polymerase<sup>2</sup>.

Pure seminalplasmin was prepared as described in the accompanying paper<sup>1</sup>. Figure 1 shows the effect of seminalplasmin on the transcription of various templates by *E. coli* RNA polymerase (holoenzyme), following a 12–30-min incubation of seminalplasmin with the polymerase alone (Fig. 1A–H) or with the template (Fig. 1G, H). With a polymerase concentration of 25  $\mu\text{g ml}^{-1}$  (in some cases, 50  $\mu\text{g ml}^{-1}$ ), the concentration of seminalplasmin required, in  $\mu\text{g ml}^{-1}$ , for 50% inhibition of the transcription of the various templates, following incubation of seminalplasmin with the polymerase for 30 min (15 min for poly(dI-dC)), was: poly(dA) 50; poly(dT) 50; poly(dA-dT) 50; poly(dG-dC) 100; poly(dI-dC) 100; poly(dA)-poly(dT) 30; T7 DNA 25; and calf thymus (CT) DNA 25. With all the templates except poly(dA-dT), poly(dG-dC) and poly(dI-dC), over 90% inhibition of transcription was obtained with seminalplasmin at 100  $\mu\text{g per ml}$  (5 nmol per ml, assuming a MW of 19,800; see ref. 1). For poly(dA-dT), poly(dG-dC) and poly(dI-dC), maximum inhibition obtained was 50–60% at a seminalplasmin concentration of 100  $\mu\text{g ml}^{-1}$ . Heating seminalplasmin for 10 min at 80 °C destroyed 90% of its transcription-inhibiting activity (Fig. 2).

The following four observations show that seminalplasmin did not possess any RNase, DNase or nonspecific protease activity that could account for its transcription inhibiting activity. (1) Seminalplasmin (130–140  $\mu\text{g ml}^{-1}$ ) did not release any acid-soluble radioactivity from—or change the polyacrylamide gel electrophoresis (PAGE) pattern of—<sup>14</sup>C-poly(rU) (37  $\mu\text{g}$  (80,360 c.p.m.) per ml) or <sup>3</sup>H-labelled total denatured *E. coli* RNA (500  $\mu\text{g}$  (360,000 c.p.m.) per ml) in 60 min at 37 °C, in conditions in which RNase A or RNase SPL (ref. 1) (8–12  $\mu\text{g ml}^{-1}$ ) converted 90–100% of the above substrates to acid-soluble material in 5–10 min. (2) Seminalplasmin (100  $\mu\text{g ml}^{-1}$ ) released no measurable acid-soluble radioactivity from <sup>3</sup>H-poly(dT) (14.8 nmol of TMP residues (1.18  $\times 10^6$  c.p.m.) per ml) on incubation for 30 min at 37 °C in the transcription buffer described in Fig. 1. (3) Seminalplasmin (50–480  $\mu\text{g ml}^{-1}$ ) did not affect the enzyme activity of  $\beta$ -galactosidase (5.5  $\mu\text{g ml}^{-1}$ ; homogeneous on PAGE) or polynucleotide phosphorylase (50  $\mu\text{g ml}^{-1}$ ), or did not release any 280 nm-absorbing or Folin reagent-positive acid-soluble material from casein (5 mg ml<sup>-1</sup>) in 20 min at 37 °C. (4) Addition of an excess (200  $\mu\text{g ml}^{-1}$ ) of bovine serum albumin to the transcription buffer (Fig. 1), did not affect the inhibition by seminalplasmin (100  $\mu\text{g ml}^{-1}$ ), of the transcription of poly(dT) by RNA polymerase (25  $\mu\text{g ml}^{-1}$ ).

The following two observations strongly suggest that seminalplasmin inhibits the transcription of various templates by binding to the RNA polymerase. (1) Increase in the concentration of RNA polymerase relieved the inhibitory effect of seminalplasmin on transcription (Fig. 3B). An increase in the concentration of the template had no such effect (Fig. 3C). (2) Incubation of seminalplasmin with the polymerase for 20–



**Fig. 1** Effect of seminalplasmin incubated with *E. coli* RNA polymerase, on the transcription of various templates. The reaction mixture contained, in 0.1 ml of Tris-HCl buffer (pH 7.8; for composition, see ref. 3): 0.15 mM of each of the substrates, UTP, GTP, CTP, ATP, and/or ITP, as required (with one of these substrates labelled with  $^{14}\text{C}$  or  $^{32}\text{P}$ ); *E. coli* RNA polymerase (holoenzyme, 5  $\mu\text{g}$  or 2.5  $\mu\text{g}$ ); template DNA; and seminalplasmin at the stated concentration. The templates used (in 0.1 ml of the incubation medium) were: T7 DNA (A), 0.244 pmol; CT DNA (B), 2.94  $\mu\text{g}$ ; poly(dA-dT) (C), 0.074  $A_{260}$  units; poly(dA-dT) (D), 0.1  $A_{260}$  units; poly(dG-dC) (E), 0.1  $A_{260}$  units; poly(dA) (F), 0.2  $A_{260}$  units; poly(dT) (G), 0.5  $A_{260}$  units; poly(dI-dC) (H), 0.05  $A_{260}$  units. In A to E, the polymerase and seminalplasmin were incubated for 30 min, DNA was added, the mixture was incubated for a further 5 min, and the reaction was started by the addition of the substrate mix. In F, the polymerase was incubated for 20 min without (a) or with (b) seminalplasmin (10  $\mu\text{g}$  in 0.1 ml). In G, either the polymerase and seminalplasmin (10  $\mu\text{g}$  in 0.1 ml) were incubated for 12 min (a), or the polymerase, seminalplasmin (10  $\mu\text{g}$  in 0.1 ml) and poly(dT) were incubated for 10 (b), 20 (c) or 30 (d) min; e, f, g and h are controls for a, b, c and d, respectively, in which the polymerase (or the polymerase and the template) was incubated without seminalplasmin. In H, the polymerase and seminalplasmin (10  $\mu\text{g}$  in 0.1 ml) were incubated for 20 min in a; the polymerase and seminalplasmin (10  $\mu\text{g}$  in 0.1 ml) and poly(dI-dC) were incubated for 15 min in b; and the polymerase alone was incubated for 20 min in c which served as the control for a and b. The time of reaction (6 min in A; 10 min in B-E; and the time given on the x-axis in F-H) was counted from the time of the addition of the substrate mix. The substrate mix contained all the four nucleoside triphosphates in the case of A and B; ATP and UTP in C and D; CTP and GTP in E; UTP in F; ATP in G; and ITP and CTP in H. The labelled precursors used were:  $^{14}\text{C}$ -UTP (2,808 c.p.m.  $\text{nmol}^{-1}$ ) in A, B, C (graph a) and F;  $^{14}\text{C}$ -ATP (2,776 c.p.m.  $\text{nmol}^{-1}$ ) in C (graph b), D and

G; [ $\alpha$ - $^{32}\text{P}$ ]CTP (24,000 c.p.m.  $\text{nmol}^{-1}$ ) in E and H. The concentration of the polymerase was 2.5  $\mu\text{g}$  in 0.1 ml in A-D; and 5  $\mu\text{g}$  in 0.1 ml in E-H. All incubations were carried out at 37  $^{\circ}\text{C}$ . A 20- $\mu\text{l}$  aliquot was withdrawn from the reaction mixture, spotted over a streak of glacial acetic acid on a  $10 \times 2$  cm strip of Whatman no. 3 filter paper, and chromatographed for 45 min in ethanol: ammonium acetate (1 M) (1:1) solvent. A 1-cm strip was cut along the original spot and the radioactivity in it measured; this radioactivity was taken as the measure of RNA synthesis<sup>4</sup>. An appropriate control, without seminalplasmin, was run in every case; the radioactivity (in c.p.m.) contained in RNA (20  $\mu\text{l}$  of the incubation mixture) in the control samples was: A 1,500; B 1,256; C(a) 1,468; C(b) 243; D 2,556; E 927. The same batch of RNA polymerase was used in all experiments described here. Its specific activity was 16,000 units per mg assayed with T7 DNA as the template, and it was 95% pure as judged by SDS-polyacrylamide gel electrophoresis; it contained 1 equivalent of the  $\sigma$  subunit. An  $A_{260}$  of 1 was taken to be equivalent to 1 mg of seminalplasmin.

30 min before the start of transcription was necessary for optimal inhibition of transcription (Fig. 1). Very much lower inhibition was obtained when seminalplasmin was added at the same time at which the transcription was started (Table 1). The inhibition was virtually abolished if seminalplasmin was added after (Fig. 3D)—or incubated with the template before (Fig. 3A)—the transcription was started.

That seminalplasmin indeed is bound tightly to RNA polymerase was shown by the following experiment. A mixture of the two proteins incubated for 30 min at 37  $^{\circ}\text{C}$  (as in Fig. 1) was chromatographed on a column of Sephadex G-75, on which column RNA polymerase and seminalplasmin, when chromatographed separately, were widely separated. RNA polymerase incubated with seminalplasmin (RP-S) was eluted in the

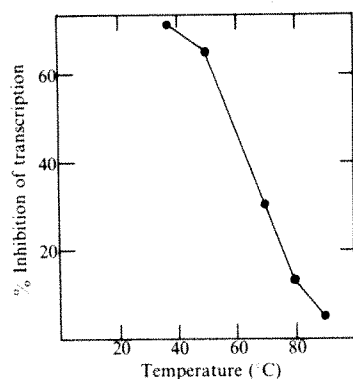
exclusion volume, like RNA polymerase incubated in the absence of seminalplasmin (RP) (Fig. 4). SDS-PAGE runs of the RP-S fractions showed that they contained seminalplasmin (Fig. 5). The subunit pattern, on SDS-PAGE, of RNA polymerase was not altered on binding seminalplasmin, showing that seminalplasmin did not exhibit any specific proteolytic activity towards the polymerase, or remove from it any of its subunits such as the  $\sigma$  factor (Fig. 5). In accord with these observations, incubation of seminalplasmin (50  $\mu\text{g ml}^{-1}$ ) for 1 h with an excess of RNA polymerase (200  $\mu\text{g ml}^{-1}$ , a concentration high enough to overcome the inhibition of transcription by seminalplasmin see Fig. 2B), did not lead to any loss in the transcribing ability of the polymerase.

As expected, the transcribing ability of RP-S, assayed on

**Table 1** Effect of seminalplasmin on transcription of various templates by *E. coli* RNA polymerase

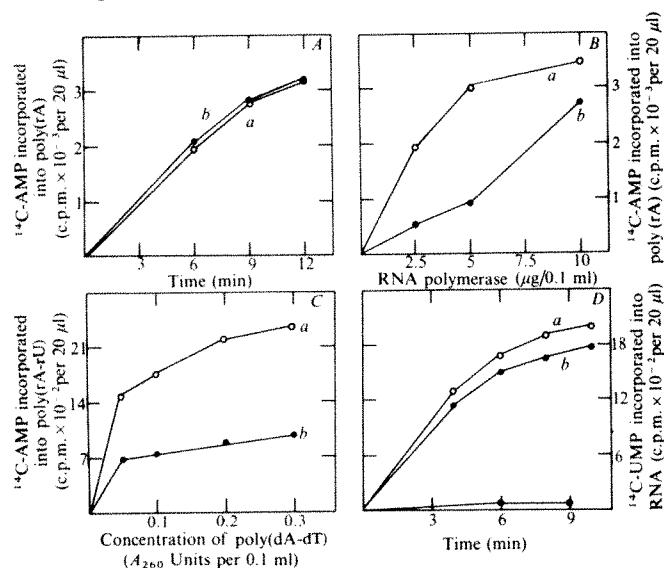
Template		Substrate	(c.p.m. $\text{nmol}^{-1}$ )	% Inhibition of transcription by seminalplasmin at:			
Description	Concentration			50 $\mu\text{g ml}^{-1}$	100 $\mu\text{g ml}^{-1}$	150 $\mu\text{g ml}^{-1}$	200 $\mu\text{g ml}^{-1}$
T7 DNA	3.7 pmol	$^{14}\text{C}$ -ATP	1,640	13	13	25	45
CT DNA	29.4 $\mu\text{g}$	$^{14}\text{C}$ -ATP	1,640	0	16	22	27
Poly(dA-dT)	2 $A_{260}$ units	$^{14}\text{C}$ -ATP	1,640	0	0	15	22
Poly(dA)·poly(dT)	2 $A_{260}$ units	$^{14}\text{C}$ -ATP	2,776	4	7	—	12
Poly(dA)	2 $A_{260}$ units	$^{14}\text{C}$ -UTP	2,833	0	0	0	0
Poly(dT)	4.8 $A_{260}$ units	$^{14}\text{C}$ -ATP	2,576	16	22	26	38
Poly(dG-dC)	1 $A_{260}$ unit	[ $\alpha$ - $^{32}\text{P}$ ]CTP	24,000	0	0	0	0
Poly(dI-dC)	0.5 $A_{260}$ unit	[ $\alpha$ - $^{32}\text{P}$ ]CTP	24,000	0	0	0	0

The effects of seminalplasmin on transcription were studied as in Fig. 1 in a total reaction volume of 0.1 ml, with the exception that seminalplasmin was added at the same time as the template, and the mixture preincubated only for 5 min before the reaction was started by the addition of substrate. The transcription was continued for 10 min [9 min for T7 DNA and poly(dT)] after the addition of the substrate mix, and 20- $\mu\text{l}$  aliquots were counted for estimation of radioactivity in RNA, as in Fig. 1. The final concentration of RNA polymerase in the incubation mixture was 50  $\mu\text{g ml}^{-1}$ .

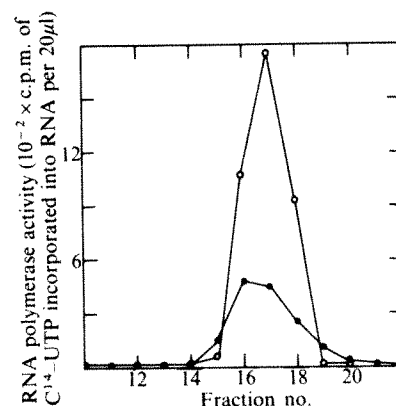


**Fig. 2** Effect of heat on the transcription-inhibiting activity of seminal-plasmin. Seminalplasmin (8  $\mu$ g), in 75  $\mu$ l of Tris-HCl buffer (Fig. 1), was heated for 10 min at the stated temperature and chilled in ice. RNA polymerase (2.5  $\mu$ g in 5  $\mu$ l of buffer) was added and the mixture incubated for 30 min. T7 DNA (0.244 pmol; 10  $\mu$ l) was added, the mixture further incubated for 5 min, and the transcription started by the addition of the substrate mix (10  $\mu$ l) and carried out for 9 min, as in Fig. 1.  $^{14}$ C-UTP was used as the labelled substrate and the inhibition of transcription measured as described in Fig. 1.

poly(dA-dT) (Fig. 4) and poly(dA), was much lower than that of RP. Seminalplasmin apparently binds to *E. coli* RNA polymerase with high affinity: the transcription-inhibitory activity of the RNA polymerase-seminalplasmin complex formed after a 20-min incubation as in Fig. 1, was not affected on incubation of the complex for 20 min in the presence of 1 M NaCl.



**Fig. 3** A, Effect of incubation of the template with seminal plasmin on transcription. Poly(dT) (0.05  $A_{260}$  units) was incubated without (a) or with (b) 10  $\mu$ g seminal plasmin for 20 min, RNA polymerase (5  $\mu$ g) was then added, the mixture was further incubated for 5 min, and the reaction was started by the addition of  $^{14}$ C-ATP; 20- $\mu$ l aliquots were taken out at various times for measurement of radioactivity in RNA. The other details were as in Fig. 1. B, Effect of increasing the concentration of RNA polymerase on the inhibition of transcription of poly(dT) by seminal plasmin. RNA polymerase was incubated without (a) or with (b) seminal plasmin (5  $\mu$ g) for 12 min, poly(dT) (0.025  $A_{260}$  units) was then added, the mixture was further incubated for 5 min, and the reaction was started by the addition of  $^{14}$ C-ATP and carried out for 9 min. The other details were as in Fig. 1. C, Effect of increasing the concentration of the template on the inhibition of transcription of poly(dA-dT) by seminal plasmin. RNA polymerase (2.5  $\mu$ g) was incubated without (a) or with (b) seminal plasmin (5  $\mu$ g) for 30 min, the stated amount of poly(dA-dT) was then added, the mixture was incubated for a further 5 min, and the reaction was started by the addition of UTP and  $^{14}$ C-ATP and carried out for 6 min. Other details were as in Fig. 1. D, Effect of addition of seminal plasmin on the transcription of T7 DNA. The transcription was started as in Fig. 1 but without incubation with seminal plasmin; the reaction mixture contained 2.5  $\mu$ g of RNA polymerase. After 2 min, 10  $\mu$ g seminal plasmin was added in b (but not in a which served as the control), and the transcription continued. In c, 10  $\mu$ g seminal plasmin and 2.5  $\mu$ g RNA polymerase were incubated for 30 min. Other details were as in Fig. 1. In all the figures, A-D, the reaction was carried out in 0.1 ml and 20  $\mu$ l of the reaction mixture was used for estimation of radioactivity in RNA as described in Fig. 1.

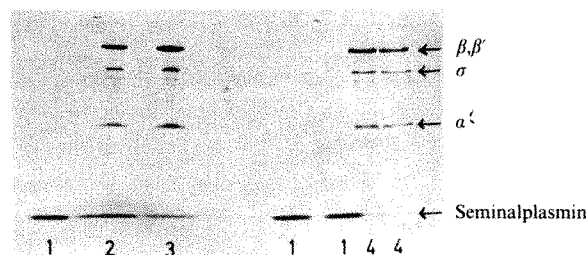


**Fig. 4** Chromatography of the RNA polymerase and seminal plasmin complex on Sephadex G-75. RNA polymerase (20  $\mu$ g) was incubated without (○) or with (●) seminal plasmin (40  $\mu$ g) in Tris-HCl buffer (Fig. 1) in a total volume of 50  $\mu$ l, for 30 min at 37°C; 40  $\mu$ l of the mixture was then loaded on a Sephadex G-75 column (1  $\times$  9 cm). The column was eluted with the Tris-HCl buffer and 80  $\mu$ l fractions collected. The polymerase activity in each fraction (10- $\mu$ l aliquot) was assayed as in Fig. 1, in a total volume of 0.05 ml using poly(dA-dT) as the template;  $^{14}$ C-UTP (4,000 c.p.m. nmol $^{-1}$ ) and ATP were used as the substrates, and the reaction was carried out for 10 min after the addition of the substrate mix.

That seminal plasmin inhibits the formation of the first inter-nucleotide linkage (the initiation step in transcription), at least in the case of certain promoters in T7 DNA recognised by the RNA polymerase *in vitro*, was shown by the following experiment done essentially as described in Fig. 1. RNA polymerase (25  $\mu$ g ml $^{-1}$ ) was incubated for 30 min without seminal plasmin (the control), or with seminal plasmin 50  $\mu$ g ml $^{-1}$ ; T7 DNA (1.2 pmol ml $^{-1}$ ) was then added, the incubation continued for another 10 min, and the transcription started by addition of a mixture of ATP and  $^{14}$ C-UTP. After allowing transcription for 30 min, a 20- $\mu$ l aliquot from each sample was subjected to ascending chromatography on a paper strip (18  $\times$  2 cm) in water:saturated ammonium sulphate solution:2-propanol (18:80:2 v/v); 1 cm-wide strips were then cut and counted. This system allows separation of pppApU and the triphosphate precursors used<sup>3</sup>. The presence of seminal plasmin in the incubation mixture caused a 34% reduction in the radioactivity found in the pppApU spot. Seminal plasmin at the concentration used in this experiment inhibited transcription of T7 DNA by 70–85%.

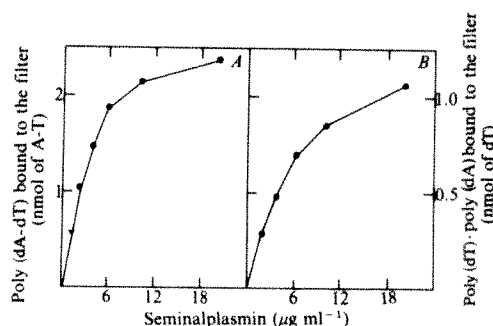
The presence of the template from the start of the incubation mixture caused little change in the inhibitory effect of seminal plasmin on the transcription of templates such as poly(dT), but significantly reduced the inhibitory effect for other templates including poly(dI-dC) (Fig. 1G,H). The reasons for this difference are also not clear.

Seminal plasmin inhibited the transcription of poly(dA-dT) by RNA polymerase core enzyme to the same extent as that by the holoenzyme; no other template was tried with the core enzyme.



**Fig. 5** Fractionation by SDS-PAGE of seminal plasmin-RNA polymerase complex from a Sephadex G-75 column. The complex was formed by incubating seminal plasmin (90  $\mu$ g) with RNA polymerase (50  $\mu$ g) in 60  $\mu$ l of the transcription buffer for 30 min at 37°C, and chromatographed on a Sephadex G-75 column, as in Fig. 4. The polymerase-containing fractions 14–16 and 17–19, eluting in the exclusion volume, were pooled separately, lyophilised, and run on SDS-PAGE<sup>5</sup> using a 5% (top)–15% (bottom) discontinuous gradient slab-gel. RNA polymerase holoenzyme and seminal plasmin were run on the same gel as controls. 1, Seminal plasmin; 2, fractions 14–16 of Sephadex G-75 column; 3, fractions 17–19 of Sephadex G-75 column; 4, RNA polymerase holoenzyme.





**Fig. 6** Binding of seminalplasmin to DNA.  $^3\text{H}$ -poly(dA-dT) (2.4 nmol of dA-dT; 8,200 c.p.m.) (A) or  $^3\text{H}$ -poly(dT) plus poly(dA) (1.48 nmol of dT; 117,700 c.p.m.) (B) was incubated in 1 ml of Tris-HCl buffer (pH 7.9) containing 0.05 M NaCl and 10 mM  $\text{MgCl}_2$ , with the stated amount of seminalplasmin for 30 min at  $0^\circ\text{C}$ , and filtered through a Millipore membrane filter (0.45  $\mu\text{m}$ ). The filter was washed slowly with 5 ml of the ice-cold buffer, dried and counted.

Seminalplasmin also binds strongly to DNA (Fig. 6A, B), a maximum of 1 mol of seminalplasmin being bound per 8 base pairs in the case of poly(dA-dT). (The stoichiometry of interaction of seminalplasmin with poly(dT)·poly(dA) (Fig. 6B) could not be estimated, as the reaction mixture contained free poly(dA) in addition to the complex.) Apparently, this binding is not involved in the inhibition of transcription.

The precise mechanism, through which the binding of seminalplasmin to *E. coli* RNA polymerase leads to loss of enzyme activity remains unclear. It is also not known why seminalplasmin inhibits the transcription of certain templates such as poly(dT), T7 DNA and CT DNA, completely, while only a partial inhibition is observed with DNAs such as poly(dA-dT) containing a repeating dinucleotide sequence.

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## Low tryptophan diet decreases brain serotonin and alters response to apomorphine

RATS fed on a low tryptophan diet since weaning have lower levels of brain serotonin than rats maintained on a control diet (18% casein diet)<sup>1,2</sup>. We have used such rats to investigate the role of the serotonergic system in the regulation of a behaviour primarily controlled by dopaminergic neurotransmission—apomorphine-induced stereotyped behaviour<sup>3–5</sup>. This behaviour, which is induced by psychomotor stimulant drugs, including apomorphine and amphetamine<sup>3–6</sup>, is repetitive, preservative behaviour that can be defined as the performance of an increasing rate of responses within a decreasing number of response categories<sup>6</sup>. In the rat, this is generally manifest as repetitive sniffing and motor movements, with repetitive licking and gnawing at higher drug levels. Although the mechanisms

controlling stereotyped behaviour are primarily dopaminergic, there are other modulatory influences, for example cholinergic and noradrenergic<sup>7,8</sup>. Apomorphine is thought to act by direct stimulation of dopamine receptors in the telencephalon<sup>9,10</sup>. There is some evidence that serotonergic mechanisms may be involved in stereotypy, because the integrity of the raphe nuclei is important for the expression of the stereotypy response produced by apomorphine and other agents<sup>11</sup>. We have found that reductions in brain serotonin (5-HT) produced by diet result in decreased stereotypy after apomorphine administration.

Weanling, male Sprague-Dawley rats were housed at six per cage and given free access to food and water. Half of the rats were fed a corn-based diet containing Masa Harina as the source of protein and carbohydrate (low tryptophan rats) and the other half were fed an 18% casein diet (control rats). Each diet contained a vitamin supplement that supplied adequate amounts of niacin. The full details of the method and diets used have been described elsewhere<sup>2,12</sup>. The rats were kept on these diets for at least 6 weeks and were rehoused in individual cages for at least 2 weeks before the start of the experiments.

On test days, rats were given doses (0.1–1.0 mg per kg, subcutaneously in the neck) of apomorphine HCl. Stereotyped behaviour (SB) was rated at 5-min intervals throughout the test session by experienced observers. The rating system was as follows: 0 = normal behaviour, inactive; 1 = normal behaviour, active; 2 = bursts of stereotyped sniffing, rearing or locomotion; 3 = continuous stereotyped sniffing, rearing or locomotion over a wide area of the cage; 4 = continuous stereotyped sniffing in one part of the cage; 5 = continuous SB with intermittent licking and gnawing; 6 = continuous SB with continuous licking and gnawing<sup>13</sup>. To assess differences between groups in the intensity of stereotypy, each rat's median score was determined, and pooled scores were analysed using the information statistic<sup>14,15</sup>. To assess differences between groups in the duration of stereotypy, each rat's rating for the final 5 min of the test was evaluated (stereotypy or no stereotypy), and pooled scores were then analysed using the information statistic.

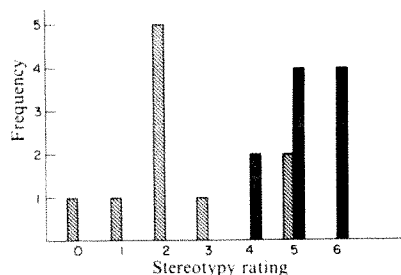
**Table 1** Number of low tryptophan or control rats showing normal behaviour or stereotyped behaviour at the final rating period of the test

Dose of apomorphine (mg per kg, s.c.)	Diet group	No. of rats showing normal behaviour	No. of rats showing stereotyped behaviour
0.1	LT	9	1
	C	5	5
0.2	LT	8	2
	C	2	8
0.5	LT	6	4
	C	1	9
1.0	LT	8	1
	C	3	7

LT, low tryptophan; C, control. At each dose of apomorphine, the differences between the two groups are significant at least at the  $P < 0.05$  level.

The apomorphine-induced stereotypy of low tryptophan rats was significantly attenuated compared with control rats (Table 1). In addition, at the 0.2-mg per kg dose of apomorphine, the low tryptophan rats showed less intense stereotypy than control rats (Fig. 1). These differences in intensity and duration of stereotypy between the two diet groups were abolished by pretreatment with L-tryptophan at 50.0 mg per kg, indicating that the reduction in stereotypy shown by low tryptophan rats was mediated by the serotonergic system.

After the behavioural experiment, a biochemical study was conducted to ensure that low tryptophan rats had decreased levels of brain 5-HT. Rats were decapitated at between 1000 h and 1200 h and brains were removed quickly, bisected midsagittally and frozen on dry ice. One half of each brain was assayed for serotonin<sup>16–18</sup>. Our results confirmed reports that



**Fig. 1** Stereotypy after administration of apomorphine (0.2 mg per kg, given subcutaneously) in low tryptophan (hatched columns) and control (black columns) rats. Categories of stereotypy rating are on the abscissa. The frequency of each rating is summed over the test session.

rats maintained on a low tryptophan diet (corn diet) for 5 weeks have lower levels of brain 5-HT than rats maintained on a control diet (18% casein diet)<sup>1,2</sup>:  $588 \pm 31$  ng g<sup>-1</sup> compared with  $675 \pm 19$  ng g<sup>-1</sup> (means  $\pm$  s.e., significant at the  $P < 0.05$  level ( $t = 2.41$ , Student  $t$  test)).

In experiments which replicated and extended the findings of the first study, we investigated the effects of a novel 5-HT agonist MK-212 and of quipazine on apomorphine-induced stereotypy in low tryptophan rats<sup>19-21</sup>. Again, the differences between the two groups in intensity and duration of stereotypy were abolished when the low tryptophan rats were treated with either MK-212 (3.0 mg per kg, intraperitoneally) or quipazine (5.0 mg per kg, intraperitoneally).

Finally, we investigated the effects of low tryptophan diet on another response thought to be mediated, at least in part, by dopaminergic mechanisms—psychomotor stimulant-induced hypothermia<sup>22,23</sup>. The pattern of results for body temperature was similar to that obtained for stereotypy, in that low tryptophan rats showed a decrease in duration of hypothermic response after intraperitoneal administration of apomorphine at 5 mg per kg compared with control rats ( $F = 6.75$ ; d.f. 1, 16;  $P < 0.025$ ). Although the mean final hypothermic response under saline was the same for both diet groups (both  $+0.2$  °C), the apomorphine-treated control rats showed a significantly greater decrease in body temperature compared with the apomorphine-treated low tryptophan rats (means  $-1.3$  and  $-0.1$  °C, respectively). These results indicate that changes in brain serotonin can affect drug-induced hypothermia.

In conclusion, it seems that the decreased stereotypy response is associated with the decreased levels of brain 5-HT of rats reared on a low tryptophan diet. These findings show that while stereotyped behaviour is primarily mediated by dopaminergic (DA) mechanisms, it can also be modulated by other neurotransmitters, including 5-HT. This DA-5-HT interaction has implications for models of psychosis based on stereotypy<sup>24</sup>. Further, this set of experiments demonstrates that diet can alter both neurotransmitter levels and behaviour.

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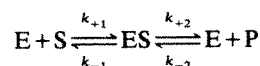
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## Frequency-dependent selection due to kinetic differences between allozymes

ENZYME polymorphisms detectable by gel electrophoresis are common in populations of animals, plants and micro-organisms<sup>1-3</sup>. Often the enzymes coded for by alternative alleles differ in their kinetic properties<sup>4,5</sup>. Although the causes of enzyme polymorphism are much disputed<sup>5-7</sup>, there has been no systematic attempt to examine the possible selective consequences of kinetic differences between allozymes. We report here a theoretical study suggesting that in some circumstances kinetic differences lead to frequency-dependent selection, which is potentially capable of maintaining balanced polymorphism.

We consider first the simplest possible model of an enzymatically catalysed reaction



where E, S and P represent enzyme, substrate and product, respectively, and  $k_{+1}$ ,  $k_{+2}$ ,  $k_{-1}$  and  $k_{-2}$  are velocity constants. If steady-state conditions prevail, and the product is not accumulating, the velocity of the reaction is

$$v = \frac{V_{\max}[S]}{K_m + [S]} \quad (1)$$

where  $V_{\max} = k_{+2}[E]$  and  $K_m = (k_{-1} + k_{+2})/k_{+1}$ . The equilibrium constant of the reaction, which is independent of the enzyme, is

$$K_{eq} = \frac{k_{+1}k_{+2}}{k_{-1}k_{-2}} \quad (2)$$

If the concentration of enzyme remains constant, the only way that  $V_{\max}$  can be increased is by an increase in  $k_{+2}$ . In most circumstances, this will increase  $K_m$  (a compensating decrease in  $k_{-1}$  is unlikely because  $k_{+2}$  and  $k_{-1}$  both reflect the stability of the ES complex, and a compensating increase in  $k_{+1}$  is unlikely because of the constraint imposed by equation (2)). Thus, if there are two allozymes differing in their kinetic constants and one of them (A) has a relatively high  $V_{\max}$  (represented as  $V_H$ ), it is also likely to have a relatively high  $K_m$  ( $K_H$ ). The lower constants of the other allozyme (a) can be represented as  $V_L$  and  $K_L$ .

This theoretical prediction is supported by the available experimental observations on the kinetic constants of alterna-

tive allozymes (Fig. 1). Although the data are sparse, they demonstrate a clear tendency for the values of  $V_{\max}$  and  $K_m$  to vary together. This being so, at a given concentration of substrate,  $[S]$ , the ratio of the velocities generated by the two allozymes will be

$$R = \frac{v_A}{v_a} = \frac{V_H(K_L + [S])}{V_L(K_H + [S])} \quad (3)$$

When  $[S]$  is very large relative to the values of  $K_m$ , this approximates to

$$R' = \frac{V_H}{V_L}$$

However, when  $[S]$  is very small relative to the values of  $K_m$ , it approximates to

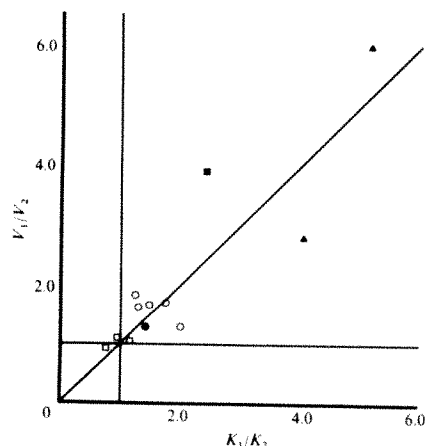
$$R'' = \frac{V_H K_L}{V_L K_H}$$

which is necessarily smaller than  $R'$ . At intermediate concentrations of  $S$ , the value of  $R$  lies between these two extremes. Thus, the relative velocity of the reaction catalysed by  $A$  is greater at high substrate concentrations than at low concentrations.

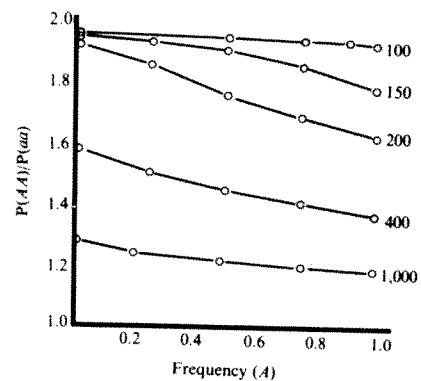
This conclusion is not restricted to a single-substrate reaction, nor to the unrealistic assumption that the reaction proceeds through only one intermediate. It follows from any system in which an increase in  $V_{\max}$  is associated with an increase in  $K_m$ . It seems that the two constants can increase together even in complex systems (see Fig. 1 and, for formulae, ref. 8).

If the rate of the reaction contributes monotonically to the relative fitness of the organism (that is, if the reaction is rate limiting for some vital process, and if the selective value is a monotonically increasing function of velocity), then individuals with the  $A$  enzyme will be relatively fitter when substrate concentrations are high than when they are low.

To study the effect on selective values of varying the numbers and frequencies of individuals, it is convenient to assume that there is in the environment a substance, either the substrate or a precursor of it, whose effective rate of uptake by the organism is dictated by the velocity of the reaction. In this case, an increase in the number of organisms acts as a proportional increase in  $[E]$ ,



**Fig. 1** Plot of relative maximum activities ( $V_1/V_2$ ) against relative  $K_m$  values ( $K_1/K_2$ ) in comparisons between allelic enzyme variants occurring in natural polymorphisms. The data come from five separate studies:  $\circ$ , ADH in *D. melanogaster* (D. Thatcher, unpublished data);  $\square$ , lactate dehydrogenase (LDH) in *Fundulus heteroclitus*<sup>24</sup>;  $\blacktriangle$ , penicillinase in *Bacillus licheniformis*<sup>25</sup>;  $\bullet$ , glucose-6-phosphate dehydrogenase in man<sup>26</sup>;  $\blacksquare$ , LDH in *Salmo gairdneri*<sup>27</sup>. The several symbols for a single enzyme represent values using different experimental conditions, either various temperatures (LDH) or substrates (ADH and penicillinase), and they are not independent of each other. All the measurements were made on purified enzymes. Symbols in the upper right and lower left quadrants support the proposed relationship.



**Fig. 2** The results of simulating the relative amount of substrate consumed, or product produced ( $P$ ), during development per individual plotted against the frequency of allele  $A$  in a population with Hardy-Weinberg genotypic proportions. The model assumes kinetic constants approximating those found empirically for the ADH polymorphism in *D. melanogaster* (ref. 13 and D. Thatcher, unpublished). The values used are  $A = Adh^F$ :  $V_{\max} = 900 \text{ pM min}^{-1}$ ,  $K_m = 0.01 \text{ M}$ ;  $a = Adh^S$ :  $V_{\max} = 450 \text{ pM min}^{-1}$ ,  $K_m = 0.005 \text{ M}$ . The heterozygote is assumed to contain only the two homodimers (because of the absence of information about the kinetic constants of the heterodimer). Development from egg to pupa is divided into 1,000 intervals of 10 min each. The amount of substrate (ethanol) consumed in each time interval is calculated by converting the rate equation (1) to a difference equation, and entering the appropriate values of substrate and enzyme concentrations. The enzyme concentrations per fly are assumed to be equal in flies of all genotypes and to increase linearly through the larval period to 100 pM per fly. The substrate concentration is increased at each interval by 0.0005 M (as if ethanol is being produced continuously by yeast in the food medium) and reduced by the amount consumed by the larvae during the previous interval. Five sets of simulations are shown that differ only by the number of larvae per vial ( $N$ ), which is given for each set.

and therefore in  $V_{\max}$  (without a concomitant increase in  $K_m$ ). Simultaneous proportional increases in  $V_H$  and  $V_L$  will not alter the values of  $R'$  and  $R''$ . However, if the supply of precursor or substrate is sufficiently limited, increases in  $V_H$  and  $V_L$  will accelerate its removal from the environment and will significantly reduce  $[S]$ . Consequently, they will shift the system away from the state represented by  $R'$ , towards that represented by  $R''$ . In other words, an increase in the number of organisms will tend to reduce the selective value of  $A$ . The selection will be density dependent.

The selection will also be frequency dependent. An increase in the number of individuals carrying the more active  $A$  enzyme will have a greater effect on  $[S]$  than an equivalent increase in the number of individuals carrying  $a$ . At high frequencies of  $A$  its selective value will be less than at low frequencies. This is frequency-dependent selection, which is potentially capable of maintaining a balanced polymorphism (see, for example, ref. 9), and follows directly from the difference between the two allozymes in their  $V_{\max}$  and  $K_m$ . Figure 2 demonstrates that the frequency-dependent effect can be appreciable.

The existence of frequency-dependent selection in the conditions assumed leads us to enquire whether it provides a general explanation for selectively maintained enzyme polymorphisms. The generality of the assumptions must, therefore, be examined critically.

The first assumption is that the rate of reaction contributes monotonically to fitness. It implies that the reaction is rate limiting to some vital process, and thus excludes non-rate-limiting enzymes. However, although at any instant only one reaction in a pathway can be rate limiting, in a changing environment several or many may be successively so. There are, of course, situations in which an 'optimal' velocity can be exceeded, when overactivity is disadvantageous. In such situations, frequency-dependent selection of the balancing kind is less likely to occur, but it is not excluded.



The second assumption is that the removal of precursor or substrate from the environment is dictated by the velocity of the reaction. This again implies that the reaction is rate limiting, but also that it takes part in a pathway concerned with the uptake of an external substance. Enzymes catalysing reactions in deeply embedded internal pathways are not expected to be subject to this kind of frequency-dependent selection.

The third assumption is that the available supply of precursor or substrate is low enough to be significantly altered by changes in the rate of the reaction. This will certainly occur when there is ecological competition for the precursor or substrate (for example, as food), because such competition necessarily implies a shortage of the resource, but it can also occur in other circumstances when the resource is not crucial to the survival of the population. The depletion, if it is to produce frequency-dependent selection, must not be merely local to the individual; it must spread to affect others. This can occur most effectively when the substance is freely diffusible, and when the organisms are crowded in a fluid environment. The frequency-dependent selection is most likely to act on organisms that obtain their food in solution (as, for example, do many microorganisms, dipteran larvae and the roots of plants). It is less likely to act on animals that ingest particulate food.

The crucial question is whether unused precursor or substrate is returned to the environment in a form available to other organisms. Among herbivores and carnivores this return may take place through the faeces, but there will usually be a delay before the substance is once more available for consumption. Such a delay will certainly tend to destabilise the system.

Both intuition and simulation suggest that the frequency-dependent effect of substrate diminution will be strongest when the concentration of substrate approximates to the  $K_m$  of the enzyme. This situation is probably common. There seem to be long-term evolutionary pressures adjusting the  $K_m$  values of enzymes towards the average concentrations of their substrates<sup>10</sup>.

Finally, it is necessary to consider how often the frequency-dependent selection will actually, rather than potentially, maintain a polymorphism. Figure 2 shows a model derived from the kinetic constant of alcohol dehydrogenase (ADH) in *Drosophila melanogaster*, in which  $V_{max}$  and  $K_m$  seem to be roughly proportional to each other. Although the selective advantage of the more active enzyme falls as its frequency increases, it is nevertheless always superior to the less active enzyme. If a genuine balance is to be achieved there must be some additional disadvantage associated with a higher  $V_{max}$ . This disadvantage may be a disproportionate increase in  $K_m$ . The more active allelic enzyme is sometimes less stable (ADH<sup>11-13</sup> and xanthine dehydrogenase<sup>14</sup> in *D. melanogaster*). Enzymes are often stabilised by the binding of substrate, so that when the concentration of substrate is low an enzyme with a higher  $K_m$  may be more liable to degradation.

The hypothesis that enzyme polymorphism can be a consequence of differences in kinetic parameters is attractive for several reasons. First, it offers an explanation of polymorphism that may be widely applicable. Second, the present model leads to several specific and testable predictions. For example, it predicts that rate-limiting enzymes should be more often polymorphic than non-rate-limiting, that enzymes with external substrates should be more often polymorphic than those intimately associated with the metabolic pathways of homeostatic organisms, and that enzymes with pervasive and freely diffusible substrates should be more often polymorphic than those with scarcer and less mobile substrates. There is already some support for these predictions. Regulatory enzymes, which are by implication rate limiting, have been claimed to have higher average levels of polymorphism than non-regulatory enzymes<sup>15</sup> (but see ref. 16). Enzymes with external variable substrates are more polymorphic than those with internal constant substrates<sup>15</sup>. Invertebrates generally have higher levels of polymorphism than vertebrates<sup>2</sup>, which have a better-developed internal homeostasis. Frequency-dependent selec-

tion has been reported to act on allozymic genotypes at two loci in *D. melanogaster* coding for enzymes with external substrates<sup>17-19</sup>.

The third attraction of the 'kinetic' hypothesis is that it offers a closer integration between the theories of enzymology, population genetics and ecology. We note, in this context, that selection for an increase in  $V_{max}$  resembles the  $r$ -selection of the ecologists<sup>20</sup>, that selection for a decrease in  $K_m$  resembles  $k$ -selection, and that our model is mathematically similar to the models of inter- and intraspecific competition put forward by Stewart and Levin<sup>21</sup> and by de Jong<sup>22</sup>. Ainsley<sup>23</sup> has suggested that kinetic differences in the ability of allelic enzymes to remove toxins can give rise to frequency-dependent selection, and his argument is similar in principle to our own.

In conclusion, it seems that biochemical differences (in  $V_{max}$  and  $K_m$ ) between allelic variants of enzymes can, in some circumstances, lead directly to frequency- and density-dependent selection. Such differences may be responsible for maintaining a significant proportion of enzyme polymorphisms, particularly among organisms taking up dissolved food.

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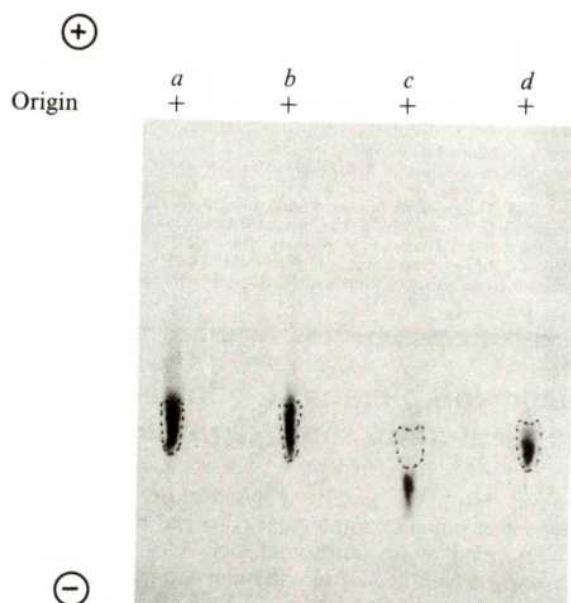
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## Amino acid substitutions in two functional mutants of yeast alcohol dehydrogenase

A METHOD has been developed<sup>1-3</sup> for selecting mutants of the structural gene for the constitutive alcohol dehydrogenase of the yeast *Saccharomyces cerevisiae*. These mutations alter but do not destroy the function of the enzyme. We report here the amino acid substitutions in two of these mutants, and examine the probable effects of these substitutions on enzyme structure and function. This is possible since the amino acid sequence<sup>4</sup>, isozyme differences<sup>5</sup> and possible subunit conformation<sup>6</sup> of the protein are known.



**Fig. 1** Autoradiogram of neutral peptides separated by electrophoresis at pH 1.9. Fluorescent peptides are indicated by dashed lines. Samples are: *a*, mutant C-40; *b* and *d*, wild-type isozyme I; *c*, mutant S-AA-5.

The mutants were isolated from strain XW517-2D, a mating type. This strain carries a mitochondrial lesion rendering it incapable of aerobic respiration (cytoplasmic petite). In common with other petite strains, the only alcohol dehydrogenase found in the cytoplasm of this strain is the constitutive ADH-I, coded by the gene *adc*<sup>7</sup>. When grande strains of yeast, which are capable of aerobic respiration, are exposed to allyl alcohol, the majority of resistant mutants are ADH-negative<sup>1,8</sup>. This prevents formation of the poisonous aldehyde acrolein from the allyl alcohol, which is by itself harmless. In contrast, petite allyl alcohol resistant mutants must continue to exhibit ADH activity, and their source of resistance has been shown in three cases<sup>2,3</sup> to be due to shifts in the intracellular concentrations of NAD and NADH. A relative increase in the latter lowers the

equilibrium concentration of acrolein in the cell. Kinetic alterations observed in the mutant enzymes affect binding constants and cooperativity, and are compatible with the observed shifts<sup>3</sup>.

Two of these mutants, S-AA-5 and C-40, were analysed. Both mutant enzymes are more basic than the wild-type form, as deduced from electrophoretic mobilities. They also exhibit kinetic differences. The enzymes were purified using affinity chromatography on AMP-Sepharose and ion-exchange chromatography on DEAE-cellulose<sup>5,9</sup>. Proteins were reduced and alkylated with iodo[<sup>14</sup>C]acetate in 6 M guanidine-HCl<sup>5</sup>. Digestion of 1 mg samples of the wild-type and mutant forms was carried out with trypsin. The mixture of peptides was separated on paper by multidimensional electrophoresis and chromatography<sup>10</sup>. The patterns were examined by autoradiography, fluorescence in UV light, and by staining with Cd-ninhydrin<sup>11</sup>.

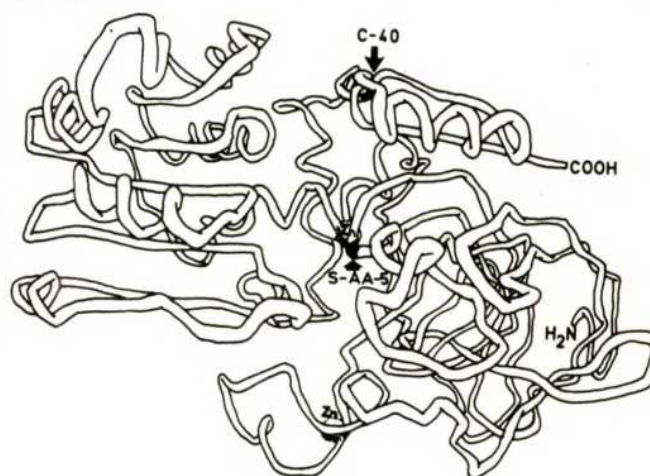
In both mutants, alterations were immediately apparent. There is only one radioactive neutral<sup>12</sup> tryptic peptide in ADH-I, and it is also the only fluorescent neutral peptide. It extends from position 39 to 59, including a histidine at position 44 (ref. 4). In S-AA-5, this peptide was absent, and instead two others were detected, a radioactive and a fluorescent fragment (Fig. 1). On staining with Cd-ninhydrin, the fluorescent peptide took a yellow colour, suggesting that it might now begin with Thr 45. This was confirmed by analysis of peptides isolated from 15 mg of the purified enzyme. The results, given in Table 1, show that the two new peptides comprise positions 39–44 (peptide 1) and positions 45–59 (peptide 2). The alteration in S-AA-5 was therefore His 44 → Arg.

The basic peptide beginning at position (315) in ADH-I, Ser-Pro-Ile-Lys<sup>4</sup>, was missing in mutant C-40, and replaced by two basic dipeptides, which on analysis (Table 1) proved to be Ser-Arg and Ile-Lys. The alteration in this mutant was therefore Pro(316) → Arg. In each mutant enzyme, the increased basicity and the extra tryptic cleavages are explained by the substitution.

The tertiary structure of the dimeric horse liver ADH has been determined to 2.4 Å resolution<sup>13</sup>. Although the yeast enzyme is a tetramer and has a highly dissimilar amino acid sequence, there is much evidence<sup>6</sup> to suggest that the two proteins have a largely conserved subunit conformation. Therefore, it is reasonable to analyse the effects of the mutations in the yeast enzyme by examining equivalent positions in the horse protein.

**Table 1** Data for the extra tryptic peptides in the mutants S-AA-5 and C-40

Mutant	S-AA-5		C-40	
	1 (radioactive)	2 (fluorescent)	1	2
Electrophoretic mobility at pH 6.5 <sup>12</sup>	0	0	-0.63	-0.63
Recovery (%)	40	40	35	30
Composition				
Cys (Cm)	0.8 (1)	—	—	—
Asx	—	2.3 (2)	—	—
Thr	—	1.6 (2)	—	—
Ser	1.1 (1)	—	1.1 (1)	—
Pro	—	1.7 (2)	—	—
Gly	1.1 (1)	1.2 (1)	—	—
Ala	—	1.0 (1)	—	—
Val	0.9 (1)	—	—	—
Ile	—	—	—	0.9 (1)
Leu	—	2.3 (2)	—	—
Tyr	0.8 (1)	—	—	—
Trp	—	+ (2)	—	—
Lys	—	0.8 (1)	—	1.1 (1)
His	—	1.7 (2)	—	—
Arg	0.9 (1)	—	0.9 (1)	—
Total:	6	15	2	2
N-terminus	Tyr	Thr	Ser	Ile



**Fig. 2** Diagram of the polypeptide backbone in horse liver alcohol dehydrogenase. The positions corresponding to the exchanges in the mutants S-AA-5 and C-40 are indicated.

Of the two mutational changes, the His → Arg substitution in S-AA-5 has the most clearcut effect. The position is shown in Fig. 2, and corresponds to Arg 47 in the horse liver enzyme. This residue forms a salt linkage with the pyrophosphate moiety of the NAD<sup>13,14</sup>. The substitution is thus one which would be expected to shift the binding characteristics of the cofactor away from those of the yeast and towards those of the liver enzyme.



As histidine may be less ionised than arginine at physiological pH, this linkage could be weaker in the yeast enzyme, resulting in a higher  $K_m$ . This is in fact the case. The  $K_m$  values for NAD for the yeast<sup>2</sup> and mammalian<sup>15</sup> enzymes are 240 and about 10 mM respectively at pH 8.8. For NADH they are 140 and about 10 mM, respectively. In mutant S-AA-5, the  $K_m$  values for both NAD and NADH have been reduced to 60 mM, consistent with the expected effect of the substitution. As the reduction in  $K_m$  is not as great as in the mammalian enzyme, other substitutions must also play a part. This is compatible with the many differences observed in other residues involved in NAD binding<sup>14</sup>. This mutant also shows a loss of positive cooperativity in the reaction ethanol  $\rightarrow$  acetaldehyde<sup>2,3</sup>. The structural reason for this is not apparent, as the nature of the interactions in the quaternary structure are unknown.

The effect of mutant C-40 is more difficult to understand. The substitution Pro (316)  $\rightarrow$  Arg occurs at a distance from both the active site and the known region of subunit interaction in the horse enzyme (see Fig. 2), yet this mutant shows alterations in both cooperativity and substrate binding. The  $K_m$  values for ethanol and acetaldehyde have been raised fivefold and twofold respectively, while those for NAD and NADH remain unchanged<sup>3</sup>. The differences appear significant even if the absolute values for the wild-type enzyme are not in agreement with those from other investigations<sup>16,17</sup>.

The substitution occurs in the catalytic domain at a point corresponding to position 346 of the horse liver enzyme. This is at a turn between an extended structure along the inter-domain cleft, and a superficial strand of a  $\beta$ -pleated sheet region<sup>13</sup>. The substitution may influence the turn. It may also increase the spacing between the domains. With unaltered central inter-domain connections, this might restrict the pocket for the substrate and hence increase the  $K_m$  values. Alternatively, the substitution may affect interactions within the quaternary structure of the enzyme. The same region shows a number of differences in closely related isozymes (positions 178, (313), (337) and (338))<sup>4,5</sup>. The lysine residue equivalent to Lys 228 in the horse liver enzyme, modification of which can increase the turnover number<sup>18</sup>, is also nearby, as well as a substitution (Ala 230  $\rightarrow$  Pro) in a variant form of human liver alcohol dehydrogenase<sup>19</sup>.

The two mutants analysed in this study are the first for which the amino acid substitutions have been determined. Many other independent allyl alcohol-resistant mutants have also been produced by selection. The present results show that such mutants do not necessarily represent changes at the same point in the molecule, not even when electrophoretic mobilities are similar (S-AA-5 showed 86% and C-40 87% of the mobility of the wild-type enzyme at pH 8.6). There is therefore strong reason to suppose that amino acid substitutions at additional positions will explain many of the other functional mutants of ADH-I, and that their determination will cast further light on the function of the molecule.

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## Human complement C4 locus is duplicated on some chromosomes

WE have described genetic polymorphism of the fourth component of human complement (C4) and postulated that it was determined by an autosomal locus close to *HLA-B* on chromosome 6 (refs 1, 2). From familial and population studies of the electrophoretic C4 patterns, O'Neill *et al.*<sup>3</sup> postulated that there are two C4 loci in all individuals, and that C4° alleles occur with high frequency at both. It was further shown that the blood groups Chido (Ch) and Rodgers (Rg) are closely associated with certain C4 types and indeed represent antigenic determinants on C4 molecules<sup>4</sup>, probably on the C4d part<sup>5</sup>. In this report we have used immunochemical methods to show that the C4 locus is definitively duplicated at least on some chromosomes. The implications are that the locus is either duplicated on all chromosomes and C4° alleles occur frequently at both, or alternatively, that the locus is duplicated on some chromosomes only.

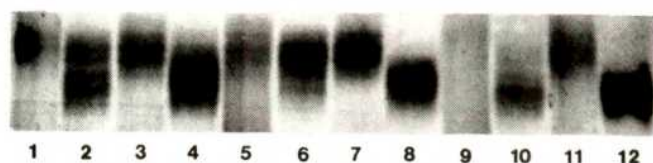
High-voltage agarose electrophoresis and immunofixation/haemolytic assay reveal several different C4 phenotypes. Familial studies of the segregation of C4 phenotypes have shown that there are three main C4 haplotype products (not phenotypes): a slow four-band 'short' pattern (S), a fast four-band short pattern (F), and a seven-band 'long' pattern including all the S and F bands (F<sub>1</sub> or FS)<sup>2</sup>. The most common of several rare haplotype products is a four-band short pattern (M) migrating slightly anodal to S. The S and M haplotypes are ordinarily Ch<sup>+</sup>, Rg<sup>-</sup>, the F haplotypes Ch<sup>-</sup>, Rg<sup>+</sup>, and the F<sub>1</sub> or FS haplotypes Ch<sup>+</sup>, Rg<sup>+</sup> (O.B. *et al.*, in preparation).

We postulated that if the C4F<sub>1</sub> (Ch<sup>+</sup>, Rg<sup>+</sup>) haplotype product was a mixture of proteins coded for by two genes, it should be possible to separate the S (Ch<sup>+</sup>) protein from F (Rg<sup>+</sup>) with an appropriate antibody. If on the other hand, the haplotype product was coded for by one gene, an antibody directed at one part of the molecule should affect the electrophoretic migration rate of both the S (Ch<sup>+</sup>) and the F (Rg<sup>+</sup>) part of the haplotype product.

C4 typing of samples was carried out as described in detail previously<sup>2</sup>. Ch and Rg typing of serum (plasma) was carried out by an inhibition assay of anti-Ch/Rg and C4 low ionic strength (LIS) coated red blood cells (RBC) using an antiglobulin reaction with IgG (R.N., in preparation). The anti-Ch serum used, serum OO, was from a patient recently transfused with several units of Ch<sup>+</sup> whole blood. Using RBC coated with C4 from Ch<sup>+</sup> serum in LIS medium, the direct agglutination titre in saline medium of this serum was 2,048, and more than 50,000 with an antiglobulin technique using pure anti-IgG. An IgG fraction of the OO serum was used in the studies described below. The serum was fractionated on a Sephadex G-200 column. The vacuum-concentrated IgG fraction gave a direct agglutinin titre exceeding 5,000 in saline against Ch<sup>+</sup> C4-coated RBC, whereas the IgM fraction was negative.

Equal volumes of heparin plasma C4S (Ch<sup>+</sup>, Rg<sup>-</sup>) and the IgG fraction of serum OO, incubated for 1 h at 37°C, showed that the IgG fraction completely removed the electrophoretic C4S pattern from the plasma. The C4 pattern of C4F (Ch<sup>-</sup>, Rg<sup>+</sup>) did not change in the same conditions.





**Fig. 1** Electrophoretic C4 patterns before and after absorption with IgG fraction of an anti-Ch antiserum. 1: Control C4F/F plasma sample; 2: F/S plasma sample; 3: F/S plasma sample absorbed; 4: FS/M plasma sample; 5: FS/M plasma sample absorbed; 6: FS/F plasma sample; 7: FS/F plasma sample absorbed; 8: M/S plasma sample; 9: M/S plasma sample absorbed; 10: FS/S plasma sample; 11: FS/S plasma sample absorbed; 12: S/S plasma sample control.

Figure 1 shows our main results. The individuals studied are members of families whose *C4/Ch/Rg* haplotypes are fully mapped from segregation in three generations. To summarise the results: The OO IgG fraction in this experiment absorbed S from S plasma, MS from MS plasma, S from F<sub>1</sub>S plasma, S from F<sub>1</sub>F plasma, S and M from F<sub>1</sub>M plasma, and S from FS plasma. It did not absorb anything from F plasma. The control experiments exclude dilution and nonspecific degradation as causes of the observed results. We have therefore shown that the *C4* locus is indeed duplicated in some individuals, that is, on either one or both chromosomes in individuals segregating for the F<sub>1</sub> pattern. We therefore now recommend a change in nomenclature to haplotypes; a person homozygous for the two genes previously called F<sub>1</sub> would be of the haplotype *FS/FS* and a person heterozygous for one F<sub>1</sub> and one F or S would be either *FS/F* or *FS/S*.

The chromosomes not coding for long patterns may, however, still have only one *C4* locus, as the guinea pig seems to have, the only animal so far studied with similar techniques<sup>6</sup>. The postulate that all human chromosomes have two *C4* loci with a high frequency of *C4*<sup>o</sup> alleles<sup>3</sup> remains unproved. This hypothesis can probably only be tested when it is possible to approach the problem on the DNA or RNA level. The present findings suggest that duplication/non-duplication of the *C4* locus may be present as a polymorphism of its own in humans. One may speculate, of course, on the possible forces behind a polymorphism of this kind. Could a duplicated *C4* locus represent an evolutionary advantage in humans, and thus represent a trait occurring increasingly frequently in the human race?

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## Sequence of the 5'-end of *Strongylocentrotus purpuratus* H2b histone mRNA and its location within histone DNA

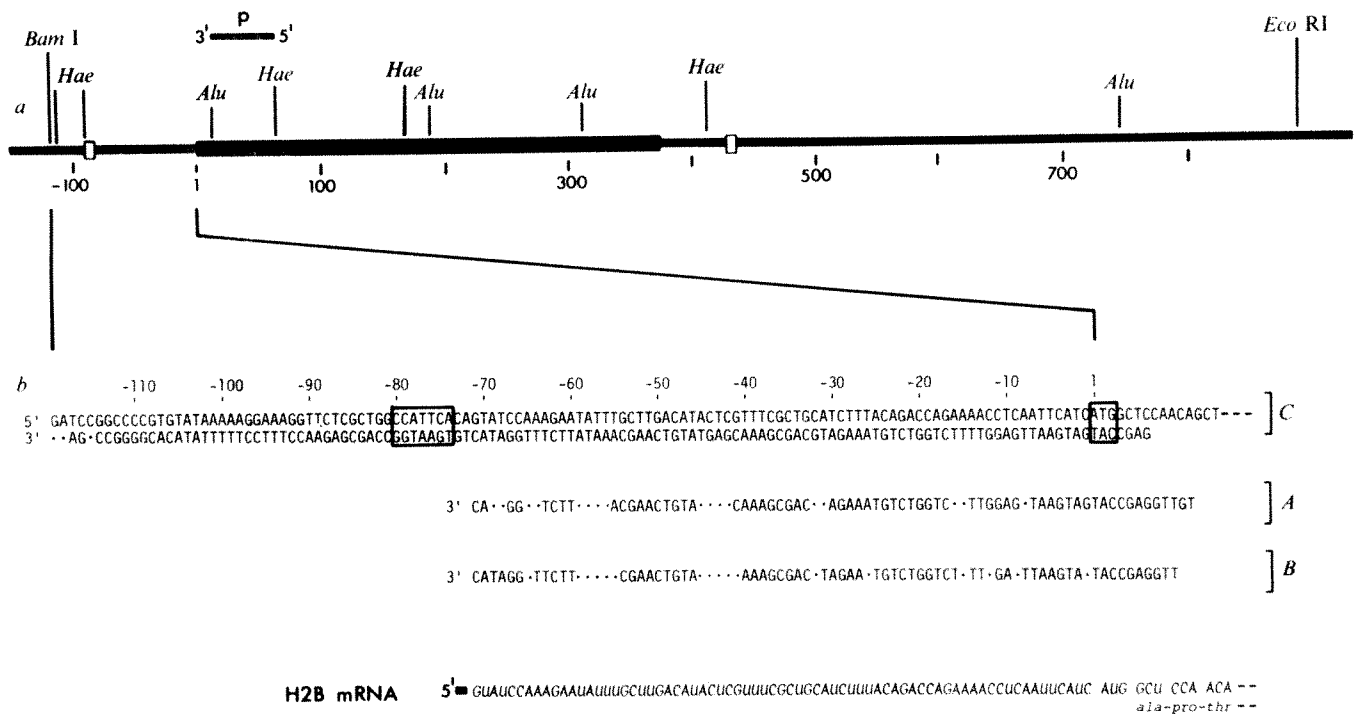
EXTENSIVE DNA sequence analysis of the cloned histone gene repeat unit from the sea urchin *Strongylocentrotus purpuratus* has identified the five histone protein coding sequences<sup>1-3</sup> (I.S., unpublished). R-loop and heteroduplex mapping strongly suggest that the sea urchin histone genes contain no intervening sequences<sup>4</sup>. In addition to the protein coding sequences the corresponding histone mRNAs each contain about 100 extra nucleotides<sup>4,5</sup>. The location of these additional sequences in relation to the coding sequences is not certain. It is of interest to map the mRNA termini within the known DNA sequence because this information may help to identify transcriptional and translational control elements. Direct nucleotide sequence analysis of RNA can be carried out by enzymatic, base-specific cleavage of end-labelled RNAs<sup>6,7</sup>. However, 5'-end labelling of mRNAs involves several enzymatic reactions ('decapping', dephosphorylation and phosphorylation) that require nuclease-free enzyme preparations<sup>8</sup>; furthermore, the RNA to be sequenced must be homogeneous. Indirect sequencing analysis of RNA can be obtained from its complementary cDNA. RNA has been sequenced in this way using a chain termination method with dideoxytriphosphates<sup>9</sup>, synthetic primers and purified mRNA templates<sup>10-12</sup>. Alternatively, end-labelled restriction fragments have been used to prime synthesis of cDNA on homogeneous<sup>13,14</sup> and partially purified<sup>15,16</sup> mRNA templates followed by either chemical<sup>17</sup> or enzymatic<sup>9</sup> sequencing of the resulting cDNA. Our approach is similar and shows that prior purification of the RNA template is unnecessary. We present here the 5'-terminal sequence of *S. purpuratus* H2b mRNA, derived from purified H2b mRNA as well as from total polysomal RNA, using the chain termination method<sup>9</sup> adapted for RNA sequencing. We also determine its location within the histone DNA sequence.

A short DNA restriction fragment, known to be located in the H2b protein coding region, was used as a primer for RNA-directed DNA synthesis. During an initial hybridisation step in which DNA/RNA hybridisation is favoured over DNA/DNA hybridisation<sup>18</sup>, the appropriate DNA strand binds to its complementary RNA sequence as a primer, and in this way selects the RNA template, which is then copied by reverse transcriptase in the presence of the four dideoxy chain terminators.

As shown in Fig. 1, we used as a primer a 51-base pair DNA fragment which is located in the NH<sub>2</sub>-terminal end of the H2b coding region and is generated by *AluI* and *HaeIII* restriction endonucleases (New England Biolabs). The 3'-end of this priming DNA strand is located 10 bases downstream from the protein initiation codon. The fragment is short enough for the elongated chain-terminated DNA to be easily separated on the sequencing gels. This DNA fragment was obtained by endonuclease digestion of pRC39 (ref. 4), a plasmid which contains a completely sequenced *BamI/EcoRI* restriction fragment (1,016 base pairs) encompassing the H2b histone gene. The digestion products were separated on 8% (1/30 cross-linked) polyacrylamide gels. This homogeneous fragment was used to prime DNA synthesis by reverse transcriptase on both purified H2b mRNA and unpurified, total polysomal RNA as described below. In parallel with the RNA template we also used a DNA template, the *BamI/EcoRI* restriction fragment. The strands of this fragment were separated by electrophoresis on a 5% (1/60 cross-linked) polyacrylamide gel<sup>19</sup> after denaturation in 50% dimethyl sulphoxide at 90 °C for 3 min (ref. 20). The faster-moving non-coding strand which has the same 5'-3' orientation as the mRNA was used as the template.

Five equivalents (10 pmol) of the double-stranded DNA primer fragment were denatured and hybridised to the





**Fig. 1** Nucleotide sequence of the 5'-end of histone H2b mRNA and its location within the DNA sequence of *S. purpuratus*. **a**, *Alu*I and *Hae*III restriction endonuclease map of the cloned *Bam*I/*Eco*RI restriction fragment containing the H2b coding region (thicker horizontal line). Open boxes indicate the locations of nucleotides common to all sequenced *S. purpuratus* histone genes. Nucleotides are numbered by reference to the first nucleotide of the initiation codon. The DNA template for reverse transcriptase is the non-coding strand of the *Bam*I/*Eco*RI fragment. The primer DNA (p) is the *Alu*I/*Hae*III fragment; its location and 3'-5' orientation is indicated. **b**, 5'-terminal nucleotide sequence of H2b mRNA (bottom line) deduced from the cDNA sequence of: (A) purified H2b mRNA; (B), polysomal RNA; (C), the non-coding DNA strand of the *Bam*I/*Eco*RI restriction endonuclease fragment. Only the positively identified nucleotides are shown. In the double-stranded DNA sequence (C) the lower strand was determined in this experiment whereas the upper strand served as a template. The initiation codon and the common nucleotides PyCATTCpu are boxed. The 'cap' structure of the mRNA is indicated by the black box.

following: (1) One equivalent (2 pmol) purified H2b mRNA, assuming that the length of the mRNA is 500 bases<sup>4,5</sup>. The mRNA was purified by hybridising total polysomal RNA extracted from early blastula stage *S. purpuratus* embryos to an excess of pRC39 DNA immobilised on cellulose as previously described<sup>21</sup>. The eluted RNA is of a single size, as judged by polyacrylamide gel electrophoresis, after 3'-end labelling with RNA ligase and <sup>32</sup>P-pCp (ref. 7 and I.S., unpublished). (2) One equivalent (2 pmol) H2b RNA in 125 µg total polysomal RNA assuming 0.25% of the polysomal RNA represents H2b mRNA. (3) One equivalent (2 pmol) single-stranded DNA of the *Bam*I/*Eco*RI restriction fragment.

DNA/RNA hybridisation was carried out in 80% formamide, 2 × SSC at 50 °C for 2 h at primer and template concentrations of 1 µM and 0.2 µM, respectively. The conditions for DNA/DNA hybridisation were as described elsewhere<sup>9</sup> except that the primer was separately denatured at 100 °C for 3 min before hybridisation. Denatured primer and single-stranded template were annealed at 68 °C for 30 min at concentrations of 0.8 µM and 0.16 µM, respectively.

The chain-terminated polymerisation reactions were carried out with reverse transcriptase as described in the legend to Fig. 2. Each set of reactions included a control mixture without dideoxy chain terminators<sup>13</sup> to identify full-length transcripts and possible artefactual bands on the sequencing gel caused by nonspecific termination during the polymerisation reactions. The <sup>32</sup>P-labelled reaction products were separated on thin 7 M urea-8% polyacrylamide (1/30 cross-linked) gels<sup>22</sup> and visualised by autoradiography.

Figure 2 shows autoradiographs of the sequencing gels. The three panels show the reverse transcriptase transcription products derived from purified DNA (a), polysomal RNA (b) and DNA templates (c). The sequences of the RNA-dependent transcripts (Figs 1bA, B, 2a, b) are identical to the sequence derived from the DNA template. The sequenced portion of the 5'-end of the H2b mRNA is therefore co-linear with the corresponding sequence of the cloned sea urchin histone genes<sup>3</sup> and does not contain any nucleotide insertions or deletions. This

co-linearity extends beyond the nontranslated leader sequence, at least to nucleotide 64 of the protein coding region (data not shown). The sequence derived from the DNA template (Figs 1bC, 2c) confirms the sequence data previously reported<sup>3</sup>.

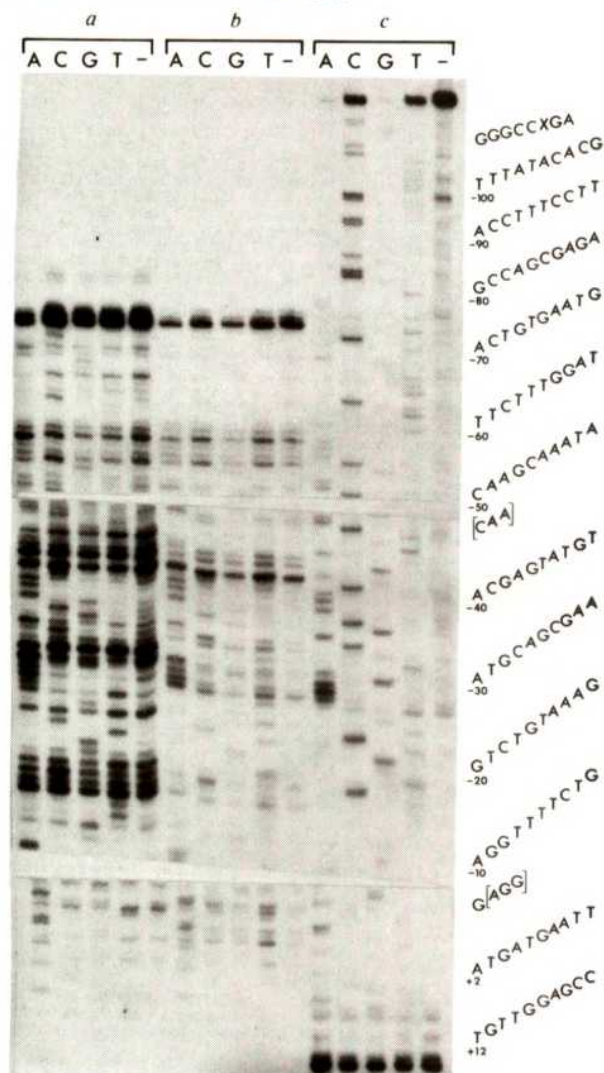
Sequences derived from the RNA template are ambiguous at certain positions, perhaps related to secondary structure or site-specific degradation of the RNA. Such nonspecific stops do not occur on the DNA template. The single-stranded DNA template which has the same 5'-3' orientation as the H2b mRNA was sequenced in parallel with the mRNA template. This side by side sequencing facilitates the determination of bases in these ambiguous positions. The DNA template, which is longer than the homologous mRNA, also serves as a control for nonspecific termination (see below). It is not necessary to determine beforehand the orientation (coding or non-coding) of the two separated DNA strands. They can both be used as templates in separate reactions and only the sequence derived from the non-coding strand serves as a reference.

It is interesting that the DNA sequence derived from purified H2b mRNA contains more nonspecific termination events than that derived from total polysomal RNA. These nonspecific stops are clustered (Figs 1b, 2a, b) and might be a result of secondary structure in the RNA template which is more pronounced in purified RNA.

Whereas the first 20 nucleotides of the RNA-dependent transcript can be read unambiguously, the first 4 nucleotides of the reaction with the DNA template are unreadable. They are probably obscured by internally labelled primer molecules. In the RNA-dependent reactions the primer is not labelled because of the presence of actinomycin D. As the purified H2b mRNA was isolated by hybridisation in vast DNA excess, contaminating DNA sequences homologous to the mRNA might be present and available as a template. Actinomycin D was thus included to ensure the exclusive use of RNA templates by reverse transcriptase.

Both RNA-dependent sequences (Fig. 2a, b) stop 71 bases upstream from the protein initiation codon (Fig. 1). Although we cannot exclude the possibility that reverse transcriptase stops





**Fig. 2** Autoradiographs of sequencing gels showing the chain-terminated transcription products of the purified H2b mRNA (a), unpurified H2b mRNA (b) and the DNA (c) templates. Electrophoresis was on 8% (1/20 cross-linked) thin polyacrylamide gels<sup>22</sup> containing 7 M urea, 100 mM Tris-borate, pH 8.3, 1 mM EDTA at 1,000 V, constant power. Sections of three gels, electrophoresed for different times, are shown, overlapping nucleotides are bracketed. The nucleotides are numbered by reference to the first nucleotide of the initiation codon. DNA/RNA hybrids were prepared as follows: 10 pmol primer DNA was dissolved in 9  $\mu$ l 90% formamide (MC/B), denatured for 5 min at 40 °C and transferred to silanised Eppendorf tubes containing 2 pmol of the dried H2b mRNA template. The solution was adjusted to 2 $\times$ SSC, 80% formamide by addition of 1  $\mu$ l 20 $\times$ SSC and hybridisation was carried out for 2 h at 50 °C. (1 $\times$ SSC = 0.15 M NaCl, 0.015 M sodium citrate). The formamide was diluted with 20  $\mu$ l 2 $\times$ SSC and the hybrids precipitated with 100  $\mu$ l ethanol, washed twice with 80% ethanol and dissolved in 12  $\mu$ l 1.66 $\times$ H buffer. (H = 50 mM NaCl, 34 mM Tris, pH 8.3, 6 mM MgCl<sub>2</sub>, 5 mM dithiothreitol). DNA/DNA hybrids were prepared in sealed capillaries in a total volume of 12  $\mu$ l 1.66 $\times$ H buffer as described in the text. The RNA sequencing reactions were carried out in a total volume of 5.25  $\mu$ l in silanised Eppendorf tubes. Each reaction contained: 2  $\mu$ l hybrids in 1.66 $\times$ H buffer; 1  $\mu$ l dNTPs [500  $\mu$ M each of dATP, dCTP, dGTP (Sigma) and 85  $\mu$ M (100 Ci mmol)<sup>-1</sup> [ $\alpha$ -<sup>32</sup>P]-TTP (Amersham)]; 1  $\mu$ l 1 $\times$ H buffer containing 3 units reverse transcriptase from avian myeloblastosis virus (Life Sciences); 1  $\mu$ l of either 1 mM ddATP, 0.5 mM ddCTP, 0.5 mM ddGTP, 0.1 mM d(d)TTP (PL Biochemicals) or H<sub>2</sub>O and 0.25  $\mu$ l actinomycin D (1 mg ml<sup>-1</sup>) in 50% ethanol. In practice, a pre-mix containing hybrids, dNTPs and actinomycin D (Sigma) was added to the individual dideoxynucleotides, followed by the enzyme addition. Incubation was for 10 min at 42 °C in a final buffer concentration of 0.8 $\times$ H. To prevent nonspecific termination due to the low [ $\alpha$ -<sup>32</sup>P]TTP concentration, 1  $\mu$ l 0.5 mM TTP in 0.8 $\times$ H buffer was added after 10 min and incubation was continued for 10 min at 42 °C. The reaction mixtures were treated with 1  $\mu$ l 1 M NaOH at 100 °C for 3 min, chilled and neutralised with 1  $\mu$ l 1 M HCl. The DNA was finally precipitated with 150  $\mu$ l ethanol after addition of 0.5  $\mu$ g yeast tRNA (PL Biochemicals) in 45  $\mu$ l 2 M NH<sub>4</sub>OAc and washed twice with ethanol. Samples were dissolved in 90% formamide, 1 mM EDTA, 0.05% xylene cyanol bromophenol blue, heated to 100 °C for 3 min, rapidly chilled and loaded on the gel. Samples containing DNA templates were prepared in the same way omitting actinomycin D and the final NaOH treatment.

because of a strong stop signal in the RNA itself, the enzyme does transcribe right through the same sequence on the corresponding DNA template. This sequence can be read clearly to the A of the *Bam*I site at position -116 (Fig. 2c). Therefore, it is very likely that the end of the sequences derived from the mRNA template represents the 5'-end of the H2b mRNA. The *S. purpuratus* histone mRNAs are capped with the structure 7mGpppX<sup>m</sup>pYp (ref. 23). As we do not know if reverse transcriptase can copy the nucleotides at the X or Y positions of the 'cap' structure, we cannot conclude that G at position -71 is the very first base of the H2b mRNA.

The 5'-terminus of the H2b mRNA determined here maps next to a short nucleotide sequence which is common to all sequenced *S. purpuratus* histone genes<sup>3</sup>. This sequence, 5' PyCATTCPU 3' (boxed in Fig. 1), is found 50-70 bases upstream from the initiation codon for H2a, H2b, H3 and H4 and nowhere else in the sequenced part of the histone gene repeat unit. As the mature H2b mRNA does not contain this septanucleotide, it is probably not a translational control element. We note also that the 5'-end of the H4 mRNA has been tentatively located adjacent to the same sequence by matching H4 mRNA oligonucleotides to the known DNA sequence<sup>2</sup>.

If we assume that the H2b mRNA is 500 nucleotides long, we can predict the location of its 3'-terminus within the DNA sequence. Interestingly, this prediction locates the 3'-terminus next to a nucleotide sequence [5' CAXGAAAGAXT 3' (boxed in Fig. 1A)] which is common to all three histone genes (H2a, H2b and H3) which have been sequenced downstream from the protein termination codon<sup>3</sup>. The function of these sequences in histone gene expression remains to be determined.

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**Note added in proof:** Recently we have formed DNA-RNA hybrids in the absence of formamide. This eliminated nonspecific chain termination events in sequencing the H2a, H3, H1 and H4 histone mRNA 5'-termini. Each of the 5'-termini coincides with the sequence 5'PyPyATTCPu3' in the genomic DNA.

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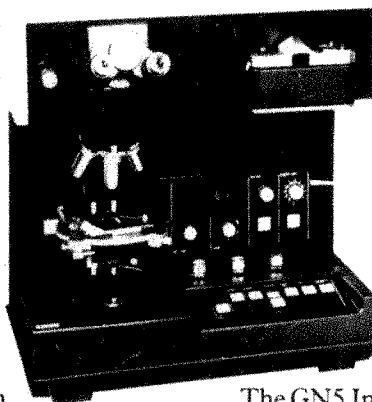
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June 1979  
0471 03504 1

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June 1979  
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0471 05678 2

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by R.S. Koff, *Veterans Administration Hospital and Boston University School of Medicine.*

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April 1979  
0471 03695 1

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## Nervous System and Hypertension

edited by P. Meyer, *Hôpital Necker, Paris* and H. Schmitt, *UER Médicale Broussais, Paris.*

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# reviews

## Still the Queen of Sciences?

Nevill Mott

*Rutherford and Physics at the Turn of the Century*. Edited by M. Bunge and W. R. Shea. Pp. 192. (McGill University Press: Montreal, 1979.) \$20.

APART from Rutherford, this book reminds the reader of the many others who were around in experimental physics at the turn of the century. In Cambridge, of course, there was J. J. Thomson, in France Becquerel, the Curies and Jean Perrin, in Germany, Röntgen, Hertz, Boltzmann and Planck, and in Holland, Zeeman and Lorentz. These men, building on the great achievements in mechanics and electromagnetism of the nineteenth century, made the discoveries which set in train the developments which led to the second explosion in our understanding, that in the decade of 1925 to 1935. This book, centered on Rutherford, is about how physicists looked at themselves and their discipline at that time, and provokes a comparison between the situation then and that in the *annus mirabilis* of quantum mechanics, and also with the present time.

The book is a series of essays by physicists and historians given on the occasion of the Rutherford Symposium at McGill University in October 1977, though at least one essay (by the late Norman Feather) had been published previously. The first, by Erwin Hiebert of Harvard, describes the state of physics at the turn of the century. First, he writes about the self-image of the physicists. They were supremely confident and physics to them was the queen of the sciences. In the nineteenth century, heat and electricity, once the domain of chemistry, had been added to physics, then called 'natural philosophy'. Now the science of the atoms, the classical domain of chemistry, was being added too. Its prestige, according to the author, was because of its generality, fundamental nature and theoretical pertinence for all the other sciences. This might have been argued at any time, but it was advocated far more openly and brazenly than ever before. This was not because of the impact on the outside world; the future of electrical technology was seen to be very promising, but apart from this the applications of chemistry to industry,



Rutherford in 1905

agriculture and war were overwhelmingly more important, in contrast to the situation today.

Apart from Einstein's Special Relativity and Planck's introduction of a quantum of radiation, the period is represented as the heyday of the experimental physicists, unexpected phenomena like X-rays, the electron and the radioactive transformation emerging one after another from their work. In this it contrasts with previous periods, with the massive theoretical achievement of Maxwell and before him Newton, and with the period from 1924-30 which saw the formulation of quantum mechanics. In this period theoretical work was inspired by an idea that did not prove successful, namely that of the mechanical Ether, which it was felt might through some singularities account for the electron and for other particles.

The experimental approach is seen as particularly English. An interesting essay by Niel Cameron (McGill) says that French thinkers, notably Pierre Duhem and Henri Poincaré, "regarded English physicists with a mixture of admiration and exasperation. They could find in them no trace of their own drive to achieve elegance, symmetry, completeness—only a passion for workable hypotheses, mechanical models and demonstrations that could

be given a readily comprehensible meaning. From the French point of view, the English had *no* theory, in the grand sense of the word, only a kind of inspired thrashing about".

The reviewer wonders if this is still a feature of English physics, in contrast with that of other communities. With frequent exchange and emigration particularly to the US there is probably less difference between one advanced country and another, and representatives of different ways of thought are to be found everywhere. Nevertheless, he remembers vividly the day when some young research workers burst into his room in the Cavendish excitedly, "Prof, come and see a moving dislocation", and showed him the little dark lines darting about on the screen of an electron microscope. "Jolly little beggars", Rutherford would have called them, and this was in the typical tradition of our country.

Perhaps also this was the end of the era when it was possible to doubt the physical existence of atoms and molecules. Ostwald was converted by cloud chamber photographs; the scientific historian Ernst Mach, founder of the Vienna Circle, perhaps never; but on the whole Rutherford's materialistic view prevailed because of its success. Rutherford, according to an essay by Stanley J. Jaki (Seton Hall), spoke about that reality with an intense commitment "as if he were defending the honour of a woman he loved". On being told by Eddington that electrons possibly were only mental concepts and had no real existence, Rutherford exclaimed, "Not exist? Not exist? Why I can see them as plainly as I see that spoon in front of me".

Most of the classic Rutherford stories are to be found in this book; and for the reviewer, there are some new ones. Of particular interest is the account of his move to McGill and the reasons for it. McGill's physics building was splendidly equipped; the donor of the institute, William Macdonald, had spared no expense. Rutherford expected—as he wrote to his fiancée, Mary Newton, in 1898—to be "practically boss man in the laboratory", but in fact this took him some time to achieve. His predecessor was Callendar, the man who revolutionised thermo-

Photo: Otto Hahn

metry, who left behind him a strong research group to carry on this kind of work. Callendar was a pupil of J. J. Thomson, an athlete, a mathematician, physicist, classicist and a fellow of the Royal Society, the first of J.J.'s pupils to rise so high. In short, when Callendar left McGill his colleagues naturally doubted that anyone so fine could be found to replace him. So at the end of his first year in McGill, Rutherford wrote to Mary Newton still waiting for him in New Zealand, "I am getting rather tired of people telling me how great a man Callendar was, but I always have the sense to agree. As a matter of fact, I don't quite class myself in the same order as Callendar, who was more an engineering type than a physicist, and who took more pride in making a piece of apparatus than in discovering a scientific truth". "This expression of snobbery"—as one essay puts it—"makes clear enough where Rutherford stood on the relative merits of pure and applied research".

And, as all the world knows, he had his way in McGill and achieved supreme success there (and incidentally the Nobel Prize for chemistry). Two things he had learned at the Cavendish had helped him. One was that one need not strive for accuracy in pioneering work. "In measuring the mechanical equivalent of heat (as did Callendar), "infinite pains are required"; in chronicling the activities of his "jolly little beggars" (ions), two-place accuracy would normally suffice. And the other was—how to form a research group. In fact, an interesting article by Lawrence Badach maintains that at McGill, Rutherford "laid the foundations of big science" in ways we would now take for granted, but perhaps not then: by the publicity he gave to his work, by the flow of research reports and publications in *Nature* and the *Philosophical Magazine*, and by the formation of research groups. Nothing succeeds like success, and it is perhaps true that at McGill Rutherford set the pattern which has carried physics to its present heights.

On reading this book, I am tempted to contrast the physics community of 1900 ( $\pm 5$ ) with the Cavendish I first knew from 1927-33, and with the situation today. In 1902 one is almost surprised to find Rutherford writing to his mother from McGill, "I have to keep going, as there are always people on my track. I have to publish my work as rapidly as possible in order to keep in the race". This feature surely remains. In 1930, as now, all over the world in all branches of science there were and are enthusiastic research groups, enjoying their work, eager to publish and to stay in the race.

But there are now many more races. Rutherford's beloved nucleus, alas, can only be pursued with megabuck machines and a degree of theoretical understanding that eludes most scientists, even physicists in other specialities. Even a branch of the subject like solid-state physics has split into many divisions: semiconductors, metals, mechanical strength, plastics and amorphous materials, each with its journals and international conferences. In contrast to 1900 and 1930, the technological importance of physics, especially in microcircuits and nuclear energy, is enormous. The computer, a child of physics, enables the behaviour of matter to be predicted in a way that would have been inconceivable

two decades ago. Whether physics is still "Queen of the Sciences" as in 1900 and 1930 is more doubtful; some of the most startling developments in the last decades have been in biology, both pure and applied. However, most physicists will feel, rightly or wrongly, that an understanding of nature is only complete when expressed in the language of physics, and in that sense now as then we can believe that ours is the supreme discipline. □

*Sir Nevill Mott was until 1971 Cavendish Professor of Physics at Cambridge. For his research work on the application of quantum mechanics to problems of solid-state physics, including non-crystalline semiconductors, he was awarded the Nobel Prize for physics in 1977.*

## What everyone needs to know about cancer immunology

*Handbook of Cancer Immunology.* Vol. 1: Basic Cancer-Related Immunology. Vol. 2: Cellular Escape from Immune Destruction. Vol. 3: Immune Status in Cancer Treatment and Prognosis (Part A). Vol. 4: Immune Status in Cancer Treatment and Prognosis (Part B). Vol. 5: Immunotherapy. Edited by H. Walters. Pp. 1867. (Garland: New York and London, 1979.) \$37.50 each volume; \$165 the set.

SOME 25 years ago it became accepted that experimental animal tumours expressed neoantigens which elicited immune responses in the autochthonous host, or as its nearest approximation syngeneic hosts receiving transplanted tumour, and these responses could control tumour growth. Since then there has been an enormous growth of research into many aspects of the immunological recognition of malignant cells. Today this approach to cancer is at a stage where there is a need to review the concepts developed over the past years, rejecting those which no longer seem tenable and consolidating those deemed worthy of further study. Indeed critics will say the time has come for tumour immunology to substantiate the concept that host immune responses really do play a significant role in controlling cancer development.

Against this background, these five volumes attempt to provide an overview of basic developments in cancer immunology. This begins (volume 1) with a series of chapters on basic cancer immunobiology, and volume 2 deals exclusively with mechanisms by which tumours may escape immune destruction. Many of the individual

articles are excellent, providing short informative reviews of topics of particular interest to the authors. Nevertheless, one is left to draw one's own conclusions about the basic concepts of tumour immunology. For example, one would have expected volume 1 to provide a critical assessment of the relative merits of chemically-induced and virus-induced tumours compared with naturally arising animal tumours as models for human cancer. In fact this problem is not considered until later (volume 3) in reviewing the role of macrophages in resistance to cancer. The mechanisms by which tumours may escape immune destruction are considered in some considerable detail (volume 2) and this topic is further reviewed in volume 4. Altogether these articles provide a comprehensive survey of humoral factors such as circulating tumour antigen and antigen-antibody complexes which may interfere with cell-mediated immunity to tumour cells. But what of suppressor cells and factors released by these cells? Apart from their consideration in one particular animal system, there is no clear statement of opinions as to whether these cells do or do not play a decisive role in modulating immunity in tumour-bearing animals.

Turning next to human cancer, volumes 3 and 4 contain a series of articles presenting studies on specific types of malignant disease. The objective here is to review evidence that the selected human cancers do elicit immune responses in the patient. Here the selection of human tumour types for review and the order of presentation is somewhat puzzling. In reviewing studies with solid tumours and leukaemias the critical evaluation of methods for detecting cell-mediated and humoral immune responses is presented in volume 4, but this is preceded in volume 3 with chapters dealing with specific examples such as malignant melanoma and cancer of the



## Health and the Family

edited by C. Wood

June/July 1979, xiv + 240pp., £11.60/\$24.50 0.12.8089.118505 (Published jointly with Grune and Stratton)

This volume deals in an unusually comprehensive way with one of the major human problems of this century, the changing status of the family. A wide range of experts from the United States and Britain consider this problem, its causes and its effect. It is important for the health professions and in particular the physician to understand the nature of this change and the importance of adapting medical education to the problems that arise. Each chapter deals with a specific aspect of the problem including the family-orientated birth, the feminist and birth-control movements, attitudes towards the aged and the effect of television on the young as well as new developments in medical education. Chapters on new educational developments in family medicine, internal medicine, paediatrics, obstetrics and psychiatry all describe the growth of a more human and personalised approach to health problems of the individual and the family. The final chapters call upon health professionals to deal more effectively with the modern epidemics of mental ill-health, alcoholism and drug abuse as well as to provide for the health needs of an increasingly aged population. This book brings together issues and problems relating health care and medical education with the sociology of the family, and illustrates the importance and unique relationship between them.

## Fantasy and Symbol

Studies in Anthropological Interpretation

edited by R. H. Hook

July 1979, xii + 304pp., £11.80/\$25.00 0.12.351250.6

Fantasy and symbol have played a part in art and life since the beginning of civilisation itself. This outstanding collection of essays in honour of George Devereux comprises papers originally presented at a symposium on 'Psychoanalysis and the Interpretation of Symbolic Behaviour' at Canberra in 1975, augmented by a number of provocative and scholarly essays by other distinguished and world-famous anthropologists on the theme of symbolic interpretation in anthropology. The collection starts with a paper by Devereux himself, arguing that fantasy and symbol, often thought to be concerned only with an unreal or imaginary world, in fact play an important part in the actions and events of daily life. Claude Lévi-Strauss examines the place taken by seeds of the bean in the myths and rites of peoples of the Ancient, Old and the New World, in an original and entertaining essay. Subsequent papers deal with symbol and fantasy in ritual and myth. This book will be of great value to anthropologists, those interested in transcultural and comparative psychology, psychiatry and psychoanalysis, or those concerned with the interpretation of myth and ritual.

Proceedings of the Serozo Symposia: No. 23

## Somatomedins and Growth

edited by G. Giordano, J. J. Van Wyk and F. Minuto

July 1979, c. 362pp., £17.60/\$37.00 0.12.285350.4

This book summarises the original papers and discussions presented at the International Symposium on Somatomedins and Growth, held at Santa Margherita, Italy, in March 1978. This symposium provided the opportunity for investigators from many different disciplines to summarise and integrate the vast amount of data which is now emerging from laboratories around the world on the chemistry and physiology of the somatomedin family of peptides. Papers were presented on the nature of somatomedins in blood, methods for their detection and the origins and physiological significance of the somatomedins. Particular emphasis was placed on the chemical nature of these compounds and their unique binding properties. Consideration was also given to the comparison of results obtained by radioreceptor assay, protein binding assay and radioimmunoassay, and to the effect of administering somatomedins to experimental animals. This book contains much that will be of interest to endocrinologists, in particular pediatric endocrinologists and diabetologists. It will also be of value to cell biologists concerned with nutritional and hormonal factors involved in cell growth.

## Speech-Hearing Tests and the Spoken Language of Hearing-Impaired Children

edited by J. Bench and J. M. Bamford

Summer 1979, xviii + 528pp., £23.50/\$45.00 0.12.088450.X

Speech-audiometry has an established place in the group of tests used by audiologists, speech and hearing therapists, educational audiologists and teachers of the deaf in order to assess a person's hearing for speech. Most speech audiometry is conducted with word lists, but some make use of lists of sentences. Until now, existing tests consisting of sentence material have been of questionable validity for testing children's speech-hearing ability. In particular, tests have been linguistically too advanced, especially with regard to the complexity of their grammar and vocabulary. This book reports the results of a large scale research project in which the primary aim was to overcome this difficulty by designing new and more appropriate speech-audiometric tests for hearing-impaired children aged 8-15 years. The background to and procedure for the research is described and discussed in detail, and the final speech tests themselves (the Bamford-Kowal-Bench (BKB) Sentence Lists for Children) are presented. The reader is given ample information on the reliability and other practical aspects of the new tests.

## Advances in Marine Biology: Volume 16

edited by F. S. Russel and M. Yonge

June 1979, xii + 436pp., £32.50/\$68.50 0.12.026116.2

Volume 16 opens with a review by R. V. Gotto of our knowledge of the systematics, occurrence, and habits of copepods living in association with marine invertebrates, many of whose actual relationships with their hosts is as yet little known. G. A. Paffenhöfer and R. P. Harris discuss the laboratory culture of wholly planktonic animals as a contribution to the understanding of food webs in the sea. The second article by A. J. Underwood reviews the ecology of intertidal gastropod molluscs from the points of view of the factors limiting their distribution and the causes of spatial and temporal variations in their abundance. Finally G. Y. Kennedy contributes an article on pigments in marine invertebrates. Marine animals and plants are often brilliantly coloured and a knowledge of the chemical composition of these pigments throws light on their evolution and physiological significance. This volume maintains the high standard of the series. With its detailed, authoritative reviews it will be of interest to fish biologists, oceanographers and biologists in general.



## Coal and Modern Coal Processing

edited by G. J. Pitt and G. R. Millward

July/August 1979, xii + c. 220pp., £8.50/\$18.00 0.12.557850.4

With the predicted exhaustion of oil reserves within decades, the importance of coal as a source of energy and chemical feedstocks is increasing rapidly. The need to develop means of processing coal more efficiently has been accompanied by the involvement of increasing numbers of scientists and engineers in research and development work on new coal processes. The purpose of this book is to provide a succinct and up-to-date introductory account of the formation, structure and main properties of coal, with brief descriptions of processes for converting it into gas, liquid fuels, coke and graphite, and for its combustion for heating and power generation. Some of the chapters include accounts of recent work that have not been published elsewhere, and sources of more detailed information are indicated throughout. It will be particularly suitable for graduates, technical staff and undergraduates in fuel science and chemical engineering, and for all those entering the coal processing industry.

## Cold Tolerant Microbes in Spoilage and the Environment

edited by A. D. Russel and R. Fuller

June/July 1979, xii + 164pp., £9.80/\$21.00 0.12.603750.7

This volume, Number 13 in the *Society for Applied Bacteriology Technical Series*, originated at a Demonstration Meeting of the society held at Brunel University's Department of Biology in Autumn 1977. The subject of the meeting was one of increasing importance to microbiologists: "*Cold Tolerant Microbes in Spoilage and the Environment*." Contributions have been brought together from experts on psychrotrophic bacteria to show their various effects on fundamental metabolism, ecology and food spoilage in the context of low temperature microbiology. An excellent survey is thus provided of the importance of psychrotrophic bacteria and of techniques involving psychrotolerant and psychrotrophic micro-organisms. For those involved in this particular area of microbiology, notably food and dairy scientists, a reference work of this standard will prove to be essential reading. It will also be of particular value to bacteriologists, cryobiologists and microbiologists, and to postgraduate and undergraduate students in these areas.

## Non-Metallic Solids: A Series of Monographs No. 1

edited by J. P. Roberts and P. Popper

## Glass Ceramics Second Edition

P. W. McMillan

July/August 1979, viii + 284pp., £16.60/\$35.00 0.12.485660.8

Since the first and widely acclaimed edition of 'Glass-ceramics' was published in 1964, the theoretical and commercial bases of this industrial science have been transformed. This second edition, greatly revised and enlarged, describes both the theoretical and practical aspects of glass-ceramics and presents a distillation of previously unpublished ideas and data. Its discussion, for example, of the formation of glass-ceramic microstructure and of microstructure-property relationships covers completely new ground. Important recent developments are surveyed, including processes for the production of very high strength materials and machinable glass-ceramics. The discussion of the applications of glass-ceramics ranges from considerations of fields in which the materials are firmly established to areas where the potential of the materials still awaits full exploitation, such as uses in nuclear power engineering and in the medical field. Throughout the text, where processes are discussed, they are illustrated by practical examples.

## The Earth: Its Origin, Structure and Evolution

edited by M. W. McElhinny

July 1979, xiv + 598pp., £36.00/\$74.50 0.12.482750.0

In this important research volume, faculty members of one of the leading research institutes in geochemistry and geophysics present reviews of the current state of knowledge of various aspects of the origins and composition of the Earth. The book begins by discussing the origins of the Earth, and goes on to consider its composition and structure, from core to crust, as deduced from seismology, geomagnetism and laboratory studies of phase transitions and physical properties at high temperatures and pressures. Geochemical aspects of the evolution of the upper mantle and crust are also discussed, including the origin of magmas, porphyry copper deposits, the isotopic evolution of granite and the distribution of rare earth elements in the continental crust. The final chapters review palaeomagnetic studies related to the past distribution of continental crust, with discussions of the geomagnetic polarity time scale and of continental drift since the Devonian. This book will be of great interest to anyone needing a review of particular aspects of the origin, structure and evolution of the Earth: students and researchers in the earth sciences, geology, geophysics and geochemistry.

## Biology and Systematics of Colonial Organisms

edited by G. Larwood and B. R. Rosen

June 1979, x + 588pp., £40.00/\$82.75 0.12.436960.X

Coloniality is a very loosely used word in biology and if it is used precisely it generally has different meanings for different specialists. A very wide range of organisms are supposed to show colonial or social behaviour, including bacteria, protozoans, coelenterates, bryozoans, echinoderms, bivalves, certain insects and gregarious vertebrates including man. Is there a common factor to these different biological associations or are there very different biological phenomena involved? In this volume specialist authors from a wide range of research backgrounds present their views on a particular group of colonial or social organisms. The editors have tried to cover as many different groups as possible, fossil and living, but inevitably the bias is on invertebrates. There is a general emphasis throughout on the likely adaptive advantages and possible evolutionary reasons for the development of coloniality. Within this general framework subjects discussed include coordination, histoincompatibility, functional morphology, chemoreception, electrophysiology and the ecological strategies of colonial growth forms.

# Numerical Analysis of Singular Perturbation Problems

edited by P. W. Hemker and J. J. H. Miller

June/July 1979, xii + 500pp., £17.50/\$37.00 0.12.340250.6

The analytical theory of singular perturbation problems is a well established area of research, which has been developing for many years. On the other hand, the numerical analysis of such problems seems to have received relatively little attention and most of this has been in the last few years. As a result of this rapid expansion of interest in recent years a conference was organised at the Catholic University, Nijmegen, The Netherlands from 30 May to 2 June 1978, to bring together mathematicians working on these problems. These published proceedings contain a collection of new explorations in the field and several applications. This volume will be of interest to research workers and lecturers in applied mathematics and numerical analysis and to all those concerned with the application of numerical methods to singularly perturbed problems such as boundary layer problems.

## The Physics of Laser Fusion

H. Motz

June/July 1979, x + 290pp., £17.50/\$37.00 0.12.509350.0.

This book provides the first synthesis of the physics of laser fusion to be accessible to the wide audience of physicists and engineers who are interested in the field but not necessarily specialists. Laser compression of matter for fusion reactions is one of the most promising approaches to the resolution of the energy problem, and research towards this end is rapidly progressing in several countries, drawing on many branches of physics. Since few specialists even can be well acquainted with each of these branches the book provides the background theory in each one and draws the various strands together to show their applications to the laser fusion problem.

## Analytical Chemistry

Second Edition

D. J. Pietrzyk and C. W. Frank

March 1979, xx + 700pp., £10.70/\$16.50 0.12.555160.6

The extensively revised version of this important textbook has a dual objective: first, to acquaint students with the fundamental principles encountered in modern chemical and instrumental methods of analysis; secondly, to enable students to master the basic quantitative skills and techniques required to perform careful measurements in the laboratory. In order to broaden the scope of the book, the authors have repeatedly used examples from related disciplines to illustrate the fundamental principles.

Journals Published by Academic Press Inc. New York

## Behavioural and Neural Biology

edited by J. L. McGaugh

Publication, Monthly. Subscription, Volumes 25-27, 1979 £112.00/\$185.25 \$157.50 (USA)

*Behavioural and Neural Biology* publishes major theoretical and review papers, experimental papers and brief communications of research findings, in all areas of neurobiology and behaviour. As an interdisciplinary journal, it includes papers concerned with neuro-anatomical, neurophysiological, neurochemical, neuropharmacological and neuroendocrinological bases of behaviour, as well as papers dealing with human and infrahuman behaviour from a biological perspective, including studies of behaviour genetics and field studies. Prompt reviewing and publishing ensure that the material is as up-to-date as possible.

## Journal of Applied Biochemistry

edited by J. J. Marshall

Publication, Bimonthly. Subscription, Volume 1, 1979 £27.30/\$45.00 \$45.00 (USA)

*Journal of Applied Biochemistry* is a new international journal devoted to the publication of papers presenting original results, review articles and other features in the field of applied biochemistry. Results of fundamental studies directly related to the design of new biotechnology, the improvement of existing biochemical processes, and the preparation, utilization, conversion or application of biological materials will also be published.

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brain and bladder. Nevertheless, these chapters present accounts of the current status of research into the immunology of human cancer and it is refreshing to read critical appraisals in which the concepts developed from experimental animal tumour studies are tested. This includes an evaluation of the *in vitro* techniques for measuring cell-mediated immunity to human tumours, the conclusion being that these are not satisfactory for detecting or monitoring immune reactivity in cancer patients. Several of the chapters in volumes 3 and 4 also consider techniques for detecting antibody responses to human tumours. This approach came into vogue in the late 1960s, but with the introduction of more sophisticated assays which were thought to detect sensitised lymphocytes it fell out of fashion. Following recognition that the cell-mediated assays are more complex than originally thought, there has been a resurgence of interest in testing for anti-tumour antibodies. In this context it is recognised that antibody assays are particularly applicable for monitoring the isolation of tumour antigens in subcellular fractions. Unfortunately the biochemistry of tumour antigens is not dealt with in any great detail in these volumes, although the potential of the serological approaches in defining tumour antigens is illustrated in a review of melanoma associated antigens.

The final volume of the series sets out to review approaches for the immunological treatment of cancer. Surprisingly a variety of non-specific

agents such as cyclic nucleotides, polianions, levamisole and neuraminidase are considered, whereas the 'well-tried' agents, BCG and C parvum, receive little attention. This approach may have been deliberate, as there are numerous reviews on cancer treatment with bacterial adjuvants. Nevertheless, this is to be regretted, as most of the clinical trials being carried out around the world have used either BCG or C parvum and so a critical appraisal of this work should have been presented as a basis for the 'newer' approaches. This volume clearly emphasises how little is known of the inter-relationships of cell-mediated and humoral immune responses generated by a developing tumour so that it is still essentially impossible to devise rational approaches to the immunological treatment of cancer.

This is an ambitious series of volumes which probably succeeds as well as could be expected considering the vagaries of the individual contributors. The series as a whole provides an overview of cancer immunology but never really comes to grip with the basic problems. These concepts could have been presented in a much more economic fashion, which would have been more appealing to the uninitiated seeking guidance into the vast literature on cancer immunology.

R. W. Baldwin

R. W. Baldwin is Professor in Tumour Biology at the University of Nottingham, and Director of the Cancer Research Campaign Laboratories, Nottingham, UK.

## Virus diseases of trees and shrubs

*Virus Diseases of Trees and Shrubs.* By J. I. Cooper. Pp. 74. (Institute of Terrestrial Ecology/National Environment Research Council: London, 1979.) £3.

THIS book is a comprehensive collation and review of the virus, virus-like and mycoplasma diseases of a wide range of the exotic and indigenous trees and shrubs found in Britain. The book does not cover *Malus*, *Prunus* and *Pyrus* species because the author felt that these plants had been adequately covered in the literature on the virus diseases of fruit trees.

Many of the virus diseases discussed were studied in other countries but they are likely to occur or be introduced into the UK. Dr Cooper also describes the main methods of studying these diseases and determining their means of spread, deleterious effects and methods of control.

It is only in recent years that this important group of diseases of trees and shrubs has been studied. This book is a very useful summation of the available information, although there are a few conspicuous omissions, for example, virus diseases of *Chaenomeles*, *Cladrastis* and *Hibiscus* species. The book is clearly written and presented, and very well illustrated with a total of 65 colour and monochrome photographs, providing a very useful identification and reference guide to those involved in the study and diagnosis of tree and shrub diseases, or those involved in the propagation and production of woody plants. At the very reasonable price of £3, it is well within the reach of students and educational establishments and will do much to make horticulturalists, plant pathologists and plant health inspectors aware of the previously neglected, but important subject of the virus diseases of trees and shrubs.

J. B. Sweet

J. B. Sweet is at Long Ashton Research Station, University of Bristol, UK.



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## Biology of the intertidal zone

*Biology of Intertidal Animals.* By R. C. Newell. Third edition. Pp. 781. (Marine Ecological Surveys: Faversham, UK, 1979.) £22.

It is almost ten years since the first edition of Professor Newell's book appeared, and during those ten years there has been a revolution in the approach to the study of intertidal animals. Much of the natural history, the ways of life and overall ecology of the commoner intertidal animals was familiar and ably put together in the first edition. What has happened since is a reappraisal of intertidal invertebrate biology by zoologists interested in the physiological and biochemical strategies used by animals in adaptation to their particular niches. Fields of particular interest which may be mentioned are those of temperature acclimation, energy budgets and facultative anaerobiosis. This new edition contains much of the original descriptions of the basic natural history, feeding mechanisms, and so on. Although these parts are brought up to date, the arrangement of the chapters is different and reflects this change in emphasis which has occurred within the last few years.

The book is cast in four sections: distribution patterns, energy acquisition, metabolic energy expenditure, and a final chapter on energy budgets. Thus, although the first edition ended with a chapter on thermal stress and desiccation, the tolerance of environmental stresses generally is brought forward and the subject is properly discussed after the initial descriptions of the intertidal environment. This includes the recent work by Trueman and coworkers on burrowing. The descriptions of faunas of special habitats has been updated by the inclusion of the discussion of the work of Fenchel, Wieser and others on the role of bacteria and other microorganisms in the ecology of fine deposits. The chapter which follows integrates the many diverse factors influencing the distribution of intertidal organisms, emphasising the fact that the distribution and occurrence of any species is the resultant of the interplay of many factors. There is a useful introduction to Alderdice's approach to this problem illustrated by various examples, including Professor Newell's own recent work on *Ligia*. In the energy acquisition section there are useful discussions of the roles of dissolved nutrients, of microorganisms in the energy equations, and of deposit and filter feeding, and it is indicative

of the modern approach that these discussions precede the description of the actual feeding mechanisms. The concept of ration and factors affecting feeding rate and the energy budgets are properly discussed after the updated descriptions of feeding.

The third section concerns energy expenditure indicated by respiratory rate and includes discussions of the interesting work of de Zwaan, Bayne, Hockachka and others on facultative anaerobiosis and of the factors affecting the rate of oxygen uptake in aerobic conditions. The final chapter attempts an integration of the various adjustments available to organisms to maintain optimal fitness.

Review papers and some recent books concerned with thermal acclimation, anaerobic pathways, enzyme biochemistry and multi-factorial analy-

sis are not easy reading even for most advanced undergraduates. Where Professor Newell deserves congratulation in his new edition is in not only providing excellent summaries in themselves but in giving sufficient background for students for an appreciation of these exciting fields of research as an introduction for their further study. The book is very well produced in a larger format than the original and is to be recommended to all who wish to understand the biology of the intertidal zone. No library serving students' needs in the biological sciences should be without it.

R. P. Dales

R. P. Dales is Professor of Zoology at Bedford College, University of London, UK.

## Rock mechanics

*Experimental Rock Deformation: The Brittle Field.* By M. S. Paterson. Pp. 254. (Springer: Berlin, Heidelberg and New York, 1978.) DM48; \$24.

LABORATORY experiments aimed at defining the mechanical characteristics of rocks under high confining pressure constitute a growth industry; people concerned with tectonic processes, earthquakes or the proper design of foundations for civil engineering projects are the customers. The subject divides naturally into the study of plastic deformation and the study of brittle fracture: the monograph under review is largely concerned with the second, but in spite of its title, it incorporates a valuable chapter on the brittle-ductile transition in rocks.

Professor Paterson, who has built up a flourishing school of rock mechanics in Canberra, has successfully set out to assemble a concise source-book. The bibliography is very extensive indeed and a scan through dates shows how modern a subject this is. David Griggs in California, building on the experimental genius of Percy Bridgman, was the father of modern rock mechanics, and his first citation dates back only to 1935. This bibliography will be of lasting value.

There are major differences between the mechanical behaviour of rocks and of metals, even brittle metals. Professor Paterson shows an impressive familiarity with the metallurgical literature and refers to it whenever it can illuminate a matter in hand; a special appendix is devoted to modern fracture mechanics, the metallurgists' creation. One special feature of rock behaviour

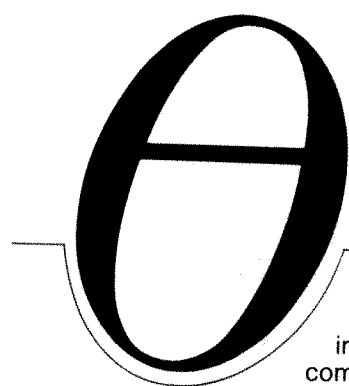
which is very clearly set out here is the linkage between incipient fracture and an anomalous enhanced dilatancy, an increase in specific volume. In the brittle-ductile transition, dilatancy again plays a major diagnostic role, and a distinction can be made between *cataclastic* flow (the microfracturing of rock followed by relative displacement of the fragments) and true crystal plasticity. The former process, but not the latter, is associated with measurable dilatancy. It seems that the dilatancy here discussed is much larger than the uniaxial microstrain preceding yield long studied by metallurgists.

Another unfamiliar subject well explained in the book is the stick-slip mechanism of frictional displacement, which has numerous implications for the behaviour of rock, both macroscopic and microscopic; presumably it serves to define the conditions of cataclastic flow. This chapter nicely supplements the long familiar treatment by Bowden and Tabor (*The Friction and Lubrication of Solids*; Clarendon/Oxford University Press: Oxford, 1950, 1964), who largely concern themselves with metallic friction. In this chapter and elsewhere in the book, Professor Paterson shows a necessary concern with the characteristics of the testing machine, stiffness in particular, which have a marked effect on the apparent fracture or flow behaviour of rock samples.

The book is exceptionally good value for money, and the reviewer now looks forward to completing his set in a few years' time with a companion volume on the plastic deformation of rocks.

R. W. Cahn

R. W. Cahn is Professor of Materials Science at the University of Sussex, Brighton, UK.



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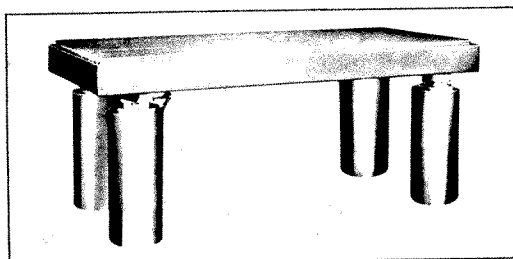
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## APPOINTMENTS VACANT

### UNIVERSITY OF NATAL

#### DEPARTMENT OF BOTANY PIETERMARITZBURG

Applications are invited from suitably qualified persons regardless of sex, religion, race, colour or national origin, for appointment to the post of

#### LECTURER

Preference will be given to candidates who have a particular interest in the field of the higher cryptogams but who would also be willing to assist in the teaching of flowering plant taxonomy and/or phytogeography.

The successful applicant will be expected to participate in the normal teaching of undergraduate courses, including field excursions, and to engage and supervise research in one of the above fields of botany.

The salary will be in the range: R8,100 to R13,200 per annum.

The commencing salary will be dependent on the qualifications and/or experience of the successful applicant. In addition, an annual vacation savings bonus is payable, subject to Treasury regulations.

Application forms, further particulars of the post and information on pension, medical aid, group insurance, staff bursary, housing loan and subsidy schemes, long leave conditions and travelling expenses on first appointment are obtainable from the Registrar, University of Natal, P.O. Box 375, Pietermaritzburg, 3200, South Africa, with whom applications, on the prescribed form, must be lodged not later than August 31, 1979, quoting reference PMB 33/79.

W179(A)

### UNIVERSITY OF CAMBRIDGE

#### PATHOLOGY DEPARTMENT POSTDOCTORAL RESEARCH BIOCHEMIST

Applications are invited from biochemists with some postdoctoral experience to fill an M.R.C. funded postdoctoral research assistantship, to collaborate on various aspects of collagen biosynthesis by cultured cells. This would involve setting up known assays for various post-translational enzymes, as well as collagen purification, chain separation and characterisation.

The appointment is for a period of 2 years in the first instance with an initial salary of £4,631 per annum, on Range 1A of the University Scale and of £4,882 for the second year.

Applications, as well as requests for further information, should be sent to Dr C. I. Levene, Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP; they should include a brief curriculum vitae, and names and addresses of 2 referees. It is hoped to fill the post by early August, 1979, or as soon as possible thereafter.

2249(A)

### PRESTON POLYTECHNIC

Applications are invited  
for the post of

#### LECTURER II IN BIOLOGY

Preference will be given to applicants with qualifications and experience in cell physiology, animal physiology or medical laboratory science.

Salary scale (under review): £4,101 to £6,558.

Application forms and further particulars may be obtained from the Personnel Officer, Preston Polytechnic, Corporation Street, Preston PR1 2TQ, to whom completed applications should be returned within 14 days from the appearance of this advertisement.

2198(A)

### SOUTHAMPTON GENERAL HOSPITAL DEPARTMENT OF HISTOPATHOLOGY MEDICAL LABORATORY SCIENTIFIC OFFICER OR JUNIOR B

The post is suitable for persons studying for H.N.C. or the special examination of the I.M.L.S. The work covers a wide range of techniques undertaken in well-equipped laboratories and there is close liaison with the Departments of Neuropathology, Electron Microscopy and University Experimental Pathology.

Further information from Senior Chief Medical Laboratory Scientific Officer, Miss M. Litchfield, telephone Southamton 777222, ext. 3956. Application forms from Mr F. Finner, Personnel Department, Southamton General Hospital, Telephone Southamton 777222, ext. 4100.

2200(A)

### SENIOR EDITOR

The International Schools Division of Macmillan Education Limited need an experienced Senior Editor to take charge of and develop a section of their schools publishing programme overseas, particularly but not exclusively in West Africa.

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Ideally we are seeking someone in their mid-twenties experienced beyond first degree level or completing post graduate work. Your experience should be in protein or enzyme chemistry involving a range of biochemical techniques. A knowledge of immunological applications would be advantageous in this specialised area of development. Working conditions are very pleasant and in addition to an excellent negotiable starting salary, relocation expenses to this most attractive area are available.

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2259(A)



# Mathematician/Physicist (or Meteorologist)

## ENVIRONMENTAL PHYSICS RESEARCH

The Environmental Physics Section, whose role at CERL is to study the physical, meteorological, and mathematical aspects of the dispersion and fate of pollution in the atmosphere, requires a mathematics or physics graduate, preferably with post-graduate experience, to undertake theoretical research on the effects of power station emissions upon climate. Of particular interest is the effect upon atmospheric temperature of carbon dioxide produced by fuel burning on a global scale and the eventual fate of this carbon dioxide in the atmosphere-ocean-biosphere system.

It is proposed to carry out this work in parallel with current research on the effect of air pollution on crops, the transport and deposition of pollution in rain and snow over large distances, and the analysis of meteorological and chemical data from long range aircraft flights. The successful candidate would be encouraged to interact with these projects whilst developing an informed position on all aspects of the carbon partitioning problem.

He/she would be working within a group of theoretical and experimental scientists studying a range of pollution problems. Excellent small and large computing facilities are available together with the latest mobile remote sensing equipment and meteorological instrumentation.

Candidates should have good honours degrees in mathematics or physics. Post-graduate study or employment experience in meteorology, fluid mechanics, or the environmental sciences would be an advantage, but we would also be interested to hear from more recently qualified graduates with an interest in entering the field.

The Laboratories are situated in a pleasant part of Surrey and offer attractive conditions of service, and facilities for the total of 800 Research and Support Staff engaged in a broad spectrum of research into the materials, technologies, and plant performance problems of the Central Electricity Generating Board.

**The initial appointment will be made within the salary range of £4300 to £7900. There are internal career prospects for advancement to research ranges of up to £10,700 and most higher graded management positions are also filled from within. These salary ranges are currently under review.**

Application forms are obtainable from the Head of Personnel Development & Services Section, Central Electricity Research Laboratories, Kelvin Avenue, Leatherhead, Surrey KT22 7SE or telephone Leatherhead 74488 Ext 363, quoting reference number **RL/5/DT**. Closing date is: **Friday 6 July 1979**. 2266(A)

## CENTRAL ELECTRICITY RESEARCH LABORATORIES

### THE UNIVERSITY OF LEEDS

#### FOOD SCIENCE DEPARTMENT

#### FOOD SCIENTIST

A FOOD SCIENTIST is required to work in the Catering Research Unit of the above Department. The work, which is supported by the Department of Health and Social Security, is connected with the application of the techniques of food science and technology to the large-scale feeding industry. Applicants should have a degree in Food Science/Technology or in a related field. The appointment will be for a fixed term ending on September 30, 1980.

Starting salary at an appropriate point on the 1B or 1A scale for Research and Analogous Staff (£3,689 to £7,145 per annum, under review).

Further information can be obtained from Mr G. Glew, Catering Research Unit, Food Science Department, The University, Leeds LS2 9JT.

Applications should be submitted to the Registrar, The University, Leeds LS2 9JT, no later than July 4, 1979, quoting reference number 72/6/D.

2227(A)

### UNIVERSITY OF STRATHCLYDE

Applications are invited from Honours graduates in appropriate disciplines for a

#### LECTURESHIP IN FORENSIC BIOLOGY (SEROLOGY)

in the FORENSIC SCIENCE UNIT in the DEPARTMENT OF PHARMACEUTICAL CHEMISTRY.

The person appointed will be expected to participate in postgraduate and undergraduate teaching, in research, and in consultancy work. Proven expertise in one or more of these areas is required and experience as a Biologist in a Forensic Science Laboratory would be a particular advantage.

Salary scale from October 1, 1979 £4,333 to £8,992 with placing according to qualifications and experience. Superannuation benefit.

Application forms and further particulars (quoting 32/79) and enclosing a self-addressed envelope (9in x 4in) may be obtained from the Academic Appointments Officer, University of Strathclyde, Royal College Building, 204 George Street, Glasgow, G1 1XW with whom applications should be lodged by July 2, 1979. 2206(A)

### THAMES POLYTECHNIC SCHOOL OF MATERIALS SCIENCE AND PHYSICS

#### DEPARTMENTAL SUPERINTENDENT GRADE 8

Applications are invited for the post of Superintendent who will be responsible for about 15 technicians who provide a complete technical support service for materials science and physics laboratories, electronics and mechanical workshops. The School has a substantial commitment to post-graduate courses and research in studies of the preparation, structure and properties of a wide range of materials involving the use of techniques such as optical microscopy, electron microscopy, X-ray analysis, electron spin resonance, ultra-violet and infra-red spectroscopy. Candidates should have substantial relevant experience and possess an appropriate H.N.D./H.N.C. qualification.

Salary scale: Technician Grade 8 £5,448 to £5,901 inclusive.

Further particulars and form of application may be obtained from the Staffing Officer, Thames Polytechnic, Wellington Street, London SE18 6PF, to whom completed applications should be returned by July 3, 1979.

2180(A)

### UNIVERSITY OF AUCKLAND New Zealand

#### SCHOOL OF MEDICINE

Applications are invited for the following vacant position. Conditions of Appointment and Method of Application are available from the Assistant Registrar (Academic Appointments), University of Auckland, or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Applications will be accepted at any time up to **AUGUST 10, 1979**.

Commencing salaries within the appropriate scale will be established in accordance with qualifications and experience up to the following maximums:

#### MEDICAL STAFF LECTURER

Maximum NZ\$17,335 per annum

The Auckland Hospital Board may make provision for a Lecturer undertaking clinical responsibilities after normal working hours to be paid an appropriate compensatory payment.

#### SENIOR LECTURER

Maximum NZ\$25,364 per annum

Senior Lecturer may be paid an appropriate allowance for clinical responsibilities.

#### ASSOCIATE PROFESSOR

Basic NZ\$27,000 per annum but in exceptional cases the Council may extend this to NZ\$30,495 per annum.

#### NON-MEDICAL STAFF LECTURER

Maximum NZ\$14,250 per annum

#### SENIOR LECTURER

Up to NZ\$16,780 per annum but in exceptional cases the Council may extend this to NZ\$18,300 per annum.

#### ASSOCIATE PROFESSOR

Basic NZ\$21,200 per annum

At present all salaries are supplemented by a General Wage Order of NZ\$365 per annum.

### PHARMACOLOGY AND CLINICAL PHARMACOLOGY

#### LECTURESHIP OR SENIOR LECTURESHIP (Medical or Non-Medical)

An especially well qualified candidate (non-medical) may be appointed at the level of Associate Professor. Applicants should have a postgraduate medical degree or qualification and/or a Ph.D. degree in Pharmacology, Clinical Pharmacology, Biochemistry or a related field and have proven ability to teach and conduct independent research. Preference will be given to those with training and active research interest in one of the following areas: pharmacokinetics (drug disposition and metabolism); passage of drugs across membranes; basic or applied autonomic, cardiovascular or respiratory pharmacology; basic or applied neurochemistry or neuropharmacology; basic or applied pharmacology related to anaesthesia. Candidates for a Lectureship or Senior Lectureship (medical) must hold a medical qualification registrable in New Zealand. It is possible that a medically qualified appointee may have clinical responsibilities in relation to the Auckland Hospital Board.

2255(A)

### LEGUME AGRONOMIST/ BACTERIOLOGIST

To participate in international field experiments to assess yield response of tropical legumes to inoculation with *Rhizobium*. Ph.D. in legume agronomy, physiology or bacteriology required. Must be prepared to travel extensively and have previous field experience with tropical legumes, and handling inoculants. Demonstrates ability to work on multi-disciplinary team would be an advantage. Applications to: Dr Jake Halliday, NITA Project of Hawaii, PO Box "O", Paia Hawaii 96779.

The University of Hawaii is an EEO/AA Employer. W175(A)





UNIVERSITY OF DUBLIN  
Trinity College

#### SCHOOL OF MATHEMATICS

Applications are invited for two Lectureships in the School of Mathematics, Trinity College, Dublin. These appointments arise as a result of a major new development in engineering education in the College. The persons appointed may be required to participate in this teaching programme.

#### LECTURESHIP IN APPLIED MATHEMATICS

Applications will be welcomed particularly from candidates with research interests in quantum field theory and its applications; or in astrophysics and cosmology or in any appropriate related fields.

#### LECTURESHIP IN PURE MATHEMATICS

Applications will be welcomed particularly from candidates with research interests in differential geometry; algebraic topology; numerical analysis or algebra, or in any appropriate related fields.

Salary scale: £4,317 to £8,487 per annum.

Appointment to each of the above posts may be made within the range £4,317 to £5,492 per annum, at a point commensurate with the qualifications and experience of the successful candidates. There is a non-contributory pension scheme.

Interested persons should, in the first instance, telephone the Staff Office, Trinity College on Dublin 772941, ext. 1678 or ext. 1775, or contact The Establishment Officer by Telex 5442 T.C.D. E1.

The closing date for receipt of completed applications will be Tuesday, July 10, 1979.

2254(A)

#### GRIFFITH UNIVERSITY

Brisbane, Australia

SCHOOL OF SCIENCE

#### READER

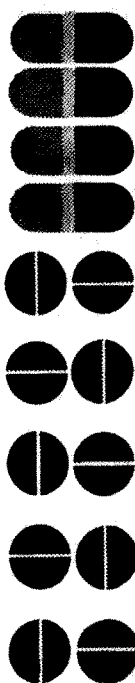
#### IN SCIENCE TECHNOLOGY AND SOCIETY

Applications are invited from qualified men and women for the senior academic appointment in the area of science technology and society (S.T.S.) within the School of Science. The appointee will have had extensive training and research experience in one or more aspects of the interaction of science with society. Applications from persons with interests in the effects of technological change and/or technology and underdevelopment would be particularly welcome. Undergraduate students in the School are required to study S.T.S. for a quarter of their time in the first year and optional courses are available in the later years. A coursework M.Sc. programme has taken its third cohort of students. The appointee will have considerable responsibilities in the teaching programmes and in the academic development of S.T.S. within the School. A Science Policy Research Centre (S.P.R.C.) has been established and the appointee will assume the position of Director of the S.P.R.C. The present salary for a Reader is £27,916. The University meets the cost of reasonable removal expenses. Applications close on August 20, 1979.

Intending applicants should obtain application procedure and further information from the Association of Commonwealth Universities (A.C.U.), Gordon Square, London WC1H 9PF.

The University reserves the right to make a fixed-term appointment.

2247(A)



## Head of Science Information Ware, Herts.

The Company is responsible for R & D of new ethical pharmaceuticals for the Glaxo Group. It is situated on two major sites at Greenford, Middlesex and Ware, Hertfordshire and employs about 1,500 staff.

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The post carries a competitive salary, fully commensurate with its attendant responsibilities, and a company car is provided. Other benefits include pension, profit sharing and productivity schemes and extensive sports facilities. Assistance with relocation will be given where appropriate.

Please send your curriculum vitae, in confidence, to: Ian Collins, Ph.D., Personnel Manager, Glaxo Group Research Ltd., Ware, Herts. SG12 0DJ.

2237(A)

## Glaxo Group Research Ltd.

#### M.R.C. CLINICAL RESEARCH CENTRE (Northwick Park Hospital)

Watford Road, Harrow, Middx. HA1 3UJ

#### POSTDOCTORAL BIOCHEMIST

The Division of Immunochemical Genetics require a Postdoctoral Biochemist, preferably with experience in enzymology and protein chemistry for investigations of the glycosyltransferase of human blood group genes. This is a limited term appointment for four or five years. Depending on age and experience salary within the range £5,631 to £8,256 inclusive London Allowance.

Application form and further details may be obtained from Mrs J. Tucker-Bull. Please quote Ref. 134/1/4223. Closing date July 14, 1979.

2223(A)

#### UNIVERSITY OF READING PROFESSORSHIP OF HORTICULTURE

Applications are invited for the Professorship of Horticulture within the Department of Agriculture and Horticulture.

The Professor will be primarily responsible within the Department for the courses and research in Horticulture, and should be prepared to foster these within the larger area covered by the Department of Agriculture and Horticulture.

The appointment will be made from a date to be arranged with the successful candidate.

Further information may be obtained from the Registrar (Room 214, Whiteknights House), The University, Whiteknights, Reading RG6 2AH, by whom applications should be received not later than August 31, 1979.

2205(A)

#### UNIVERSITY OF NAIROBI—KENYA

Applications are invited for LECTURERS in the DEPARTMENT OF CROP SCIENCE.

Applicants should be holders of Ph.D. with specialisation in either Agricultural Statistics, Weed Science, Plant Breeding or Plant Physiology. Experience in teaching and research is essential. Holders of an MSc with experience may be considered. The appointee should participate in teaching undergraduate and postgraduate courses as well as research activities. Salary scale: Lecturer K£2016 to 3984 per annum (K£1=£1.18 sterling). The British Government may supplement salaries in range £4,278 to 4,818 per annum (sterling) for married appointees and £2,730 to 3,156 per annum (sterling) for single appointees (reviewed annually and normally free from tax) and provide childrens education allowances and holiday visit passages. Family passages; SSSF or FSSU; non-contributory medical scheme; subsidized housing/housing allowance; various allowances. Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to Registrar (Recruitment and Training) University of Nairobi, P.O. Box 30197, Nairobi, Kenya by July 30, 1979. Applicants resident in the UK should also send one copy to Inter University Council, 90/91 Tottenham Court Road, London, W1P 0DT. Further details may be obtained from either address.

2239(A)

## CSIRO AUSTRALIA Postdoctoral Research Fellow

### Division of Building Research Highett, Victoria

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees 2,400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

FIELD: Planning and Management Studies.

GENERAL: The research programme of the Division includes research on planning, forecasting and technology assessment in the building industry as part of a more general programme of studies of the building industry and building development, utilizing systems and operations research techniques.

DUTIES: Carry out studies of building development and the building industry including technology assessment, industry stability studies and demand forecasting. Develop techniques to aid in planning, management and stabilization in the building industry.

QUALIFICATIONS: A PhD degree or equivalent in a relevant discipline together with demonstrable research ability. Applicants should preferably have an ability in mathematics, economics and/or computing techniques together with experience appropriate to the duties above.

SALARY: Research Scientist/Senior Research Scientist: \$A15422-\$A22405 pa.

TENURE: Term of 2 to 5 years.

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 390/613 should reach:—

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 20th July 1979.

Applications in U.S.A. and Canada should be sent to:—The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, N.W., Washington, D.C., 20036, U.S.A. 2244(A)

## MERSEYSIDE COUNTY MUSEUMS

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(£3,732 to £4,632 per annum, inc. supplement)

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Applicants for these posts, which are based at the County Museums, Liverpool, should possess a degree in an appropriate subject and/or the Museum Association Diploma. Experience of museum work or work in an associated field is desirable.

Candidates for posts (A) and (B) should have an interest in systematics and natural history, and for post (C) in military and social history, land transport or industrial archaeology. A knowledge of industrial culture and social anthropology with special interest in one geographical area is necessary for post (D).

Applications, giving details of experience and full curriculum vitae, should reach the Director, Merseyside County Museums, William Brown Street, Liverpool L3 8EN, by June 27, 1979. Tel: 051-207 0001. 2256(A)



UNIVERSITY OF DUBLIN  
Trinity College

### LECTURESHIP IN CHEMISTRY

Applications are invited for the above post in the Department of Chemistry, Trinity College, Dublin, tenable from September 1, 1979, or as soon as possible thereafter. This vacancy has arisen as a result of government-sponsored developments in engineering.

Current developments in the department include the setting up of studies of photoemission from adsorbates, and applicants with research interests in surface science or other related areas will be particularly welcome, but applications from candidates with interests related to any of the departmental fields of research will be carefully considered.

Salary scale: £4,317 to £8,487 per annum.

Appointment may be made within the range £4,317 to £5,492 per annum, at a point commensurate with the qualifications and experience of the successful candidate. There is a non-contributory pension scheme.

Interested persons should, in the first instance, telephone the Staff Office, Trinity College, on Dublin 772941, ext. 1678 or ext. 1775, or contact the Establishment Officer by Telex 5442 T.C.D. E1.

The closing date for receipt of completed applications will be Friday, July 27, 1979. 2252(A)

### UNIVERSITY OF BRISTOL LONG ASHTON RESEARCH STATION PLANT BREEDER

Required to be responsible in a small team for day-to-day organisation of an expanding strawberry breeding programme.

This will include the selection and breeding of improved varieties involving field trials at and beyond Long Ashton, investigating crop production methods, and disseminating information to advisers, growers and industry.

The work will also include some aspects of the Station's mutation breeding project on top and soft fruit plants and woody ornamentals.

Appointment in the Higher Scientific Officer (£4,101 to £5,448) or Senior Scientific Officer (£5,154 to £6,898) grade, salaries under review. Starting salary according to qualifications and experience. Non-contributory superannuation scheme.

Minimum qualifications: 2(i) Honours degree or equivalent in a botanical or horticultural subject, with at least two years' relevant post-qualifying experience. Applicants should have experience in plant breeding or biochemical/genetic techniques with a Ph.D. preferred.

Further particulars and application forms from the Secretary, Long Ashton Research Station, Bristol BS18 9AF, to whom applications should be sent by July 13, 1979. 2240(A)

### DEPARTMENT OF BIOCHEMISTRY UNIVERSITY OF NOTTINGHAM Queen's Medical Centre Nottingham NG7 2UH POSTDOCTORAL RESEARCH ASSISTANT

An M.R.C. financed position is available for three years from October 1, 1979 to work on "The Mechanism of Protein Degradation: studies on adipose tissue". A knowledge of protein chemistry, protein turnover or immunochemistry would be advantageous.

Starting salary £4,232 per annum. Candidates should write to Dr R. J. Mayer at the above address. 2177(A)

### CYCLOTRON UNIT MEDICAL RESEARCH COUNCIL

requires a physiologist or biologist investigate aspects of late radi damage to normal tissues. The is part of an extensive programme research into the relative effect X-rays and fast neutrons on no tissues and tumours at both clinical and experimental level. special concern are effects on nervous system and vascular dai and it will be an advantage if candidate has some previous experi working with either of these tissues.

The post is for 3 years. Mini qualifications are a good hon degree and a Ph.D or equivalent relevant subject.

Salary, according to age and perience, in the range £5,550 £7,129 (inclusive of London We ing), plus 6 weeks annual leave.

Please write, giving qualifica and curriculum vitae, to: Miss A Pires, M.R.C. Cyclotron Unit, I mersmith Hospital, Ducane R London, W12 0HS. Tel: 01-743 4 ext. 103. 2232(C)

### POSITION AVAILABLE APICULTURE UNIVERSITY OF GUELPH

The Department of Environm Biology, University of Guelph, been authorised to recruit a fa member to have major responsib for international programs Apiculture.

The position will be probatio at the Assistant or Associate Prof level on a tenure track.

Responsibilities of the position include the following:

- Direction of ongoing projects
- Apiculture in developing count
- Development of new projects in veloping countries and expansio existing ones to improve
- Apiculture.

- Training of students from devl ing countries.

- Participation in the teaching research programs of the De ment.

It is envisaged that the pos will be about 75% international volvement (50% directing projects 25% student training).

#### Qualifications

A Ph.D. or equivalent and field research experience in Apicul Willingness to travel extensively work in developing countries.

Salary will be commensurate experience. The minimum salary the Assistant Professor rank \$18,236, and for the Associate fessor rank \$23,055.

Contingent upon the availabil ity funds, the position will be avai January 1, 1980.

Applications should include a c plete resumé and the names of t referees and should be sent Chairman, Department of Env mental Biology, University of Guelph, Ontario, Canada N1G 2

Closing date: September 1, 1979 W174(C)

### UNIVERSITY OF STRATHCLYDE APPLIED GEOLOGY TECHNICIAN GRADE

required to undertake duties involv the operation and maintenance atomic absorption spectroph meters. Candidates with other chen laboratory experience will be gi sidered as training will be gi Applicants should hold an H.N.C equivalent qualification.

Salary Scale (including all sup ments): £3,474 to £4,056 per annu

Applications in writing, que reference GL.24, giving details of experience and qualifications, sh be made to The Personnel Off University of Strathclyde, Royal lege Building, 204 George St Glasgow G1 1XW. 2199(J)

# THE UNIVERSITY OF WESTERN AUSTRALIA

Perth

## SENIOR LECTURESHIP IN MATHEMATICS

Applications are invited for appointment as Senior Lecturer in the Department of Mathematics. The position is available from January 1, 1980, and the appointee will be expected to assume duty not later than June and preferably before March 1, 1980. Candidates should state in their applications the earliest date in 1980 on which they would be able to take up duty, if appointed.

Applications will be considered from persons with interests in the general area of probability and statistics, but some preference may be given to candidates with expertise and experience in applied statistics and consulting. If possible, the position will be filled at the level of Senior Lecturer, but a sufficiently well qualified and experienced candidate may be considered for appointment as Associate Professor.

The Department is responsible for courses in pure mathematics, applied mathematics and mathematical statistics up to Honours level, and postgraduate degrees may be taken in any of these areas. Further information concerning the Department and the research interests of its present staff may be obtained from the Chairman, Professor A. L. Blakers. Information concerning the statistical work of the Department, including statistical consulting and its possible development, may be obtained from Professor T. P. Speed.

The current salary ranges are: Senior Lecturer \$A21,180 to \$A24,687 per annum; Associate Professor \$A27,916 per annum. Benefits include superannuation similar to F.S.S.U., fares to Perth for appointee and dependent family, removal allowance, study leave and long service leave and housing loan scheme.

Applications in duplicate stating full personal particulars, qualifications and experience should reach the Acting Staffing Officer, University of Western Australia, Nedlands, Western Australia 5009, by August 18, 1979. Candidates should request three referees to write immediately to the Acting Staffing Officer, 2262(A).

## MEDICAL

### RESEARCH COUNCIL PNEUMOCONIOSIS UNIT

Llandough Hospital, Penarth  
South Glamorgan CF6 1XW

Applications are invited  
for a

Consultant grade post  
in

## RESPIRATORY MEDICINE

to carry out research into the cause, diagnosis, prevention and treatment of non-infectious extrinsic lung disease. The work includes the direction of a well staffed and equipped respiratory function research laboratory as well as the care of in and out patients at Llandough Hospital.

The successful applicant will be expected to pursue his or her own research as well as collaborate in projects involving other sections of the unit.

Applicants should be qualified to hold a consultant post in respiratory medicine and have special experience in the laboratory investigation of respiratory function. The successful applicant will be offered an unlimited appointment to the Council's clinical staff. Terms of employment will be those normal for M.R.C. clinical staff with honorary consultant sessions in the National Health Service. Salary present is £9,528 to £12,084 and compulsory pension scheme.

Applicants should contact the Director, M.R.C. Pneumoconiosis Unit, from whom further information and an application form may be obtained. Closing date: August 1, 1979. 2203(A)

## AERONAUTICAL RESEARCH LABORATORIES Australian Department of Defence

# Chief Superintendent

### Salary:

\$Aust. 34,066 (at present exchange rate £1 = \$A1.85).

### Location:

Melbourne, Australia.

### Duties:

In accordance with approved policies, undertake the management and scientific direction of the activities of the Aeronautical Research Laboratories.

In particular:

- Direct the implementation of the approved research and development programme, continuously review the programme and advise on any changes considered necessary.
  - Initiate and develop proposals for new items and/or activities for inclusion in the programme.
  - Accept responsibility as allocated for the scientific direction of major projects including extra mural activities.
  - Provide an advisory, consultative, standards and calibration service to the defence services, Australian and State Government departments and instrumentalities and industry, and direct the conduct of research and investigations in support of this service.
- Represent the Defence Science and Technology Organisation on committees, etc. as directed.

### Qualifications:

A degree in science or engineering combined with long experience in research and development. The position requires a person who has proven ability to manage scientific laboratories and to evolve research programmes to match defence needs.

The Aeronautical Research Laboratories are located at Fishermen's Bend, a suburb of Melbourne, the capital city of the State of Victoria.

Conditions of permanent appointment for the successful applicant and family include first class air travel to Australia, assistance with removal expenses and temporary rental assistance on arrival in Australia. Conditions of service include four weeks annual leave and accumulating sick leave benefits as well as a comprehensive superannuation scheme.

Detailed information relating to these conditions may be obtained from the Office of the Public Service Board Representative, Australia House, Strand, London WC2 (Telephone: 01-438 8448). Further information regarding this position can be obtained from Professor P T Fink, Chief Defence Scientist, Department of Defence, Russell Offices, Canberra A C T, Australia 2600. Applications detailing professional qualifications and experience should be forwarded to the Chief Defence Scientist by 31 July 1979. 2213(A)

# Australia

## THE UNIVERSITY OF LIVERPOOL

Walter Myers Chair of  
Parasitology

Applications are invited for the Walter Myers Chair of Parasitology, a full-time Chair in the Liverpool School of Tropical Medicine. The appointment will be made either as a clinical appointment, in the case of the successful candidate having a registrable medical qualification, or as a non-clinical appointment.

The salary will be up to £12,084 per annum (under review) in the case of a clinical appointment, or not less than £11,484 per annum (non-clinical appointment).

Applications (15 copies), together with the names of three referees, should be received not later than July 30, 1979 by the undersigned from whom further particulars may be obtained. (Candidates overseas may send only one copy by airmail.) Quote Ref RV/657/N. H. H. Burchinal, Registrar, The University, P.O. Box 147, Liverpool, L69 3BX. 2226(A)

## University College Hospital Medical School

Applications are invited for the post of

# Senior Lecturer

in Oral Microbiology (non-clinical) within the Dental School. The duties include teaching oral microbiology and assisting in the teaching of general microbiology to dental students. The successful applicant will be expected to conduct research, particularly on the bacteriology of periodontal disease and the metabolic activities of plaque organisms in dental caries.

Applications, with the names of two referees, to the Dean of Dental Studies, University College Hospital Dental School, Mortimer Market, London WC1E 6JD, within three weeks of the date of this advertisement. 2220(A)



# Experimental Scientists

## Neutron Beam Research

A new intense source of pulsed neutron beams, the spallation neutron source, is being built at the Rutherford Laboratory for use in research programmes of UK universities and polytechnics. A substantial number of neutron scattering instruments will be provided based on the time-of-flight method and outline designs have already been completed for the first seven. A wide range of equipment and techniques is involved; for example, position sensitive detectors for epithermal neutrons; high resolution choppers; sample environments controlling parameters such as temperature, pressure, magnetic field; neutron guides; neutron polarisation systems.

There are vacancies for support staff to participate in the development and provision of this equipment in the Neutron Beam Research Programme, which is expanding to meet the requirements for efficient utilisation of the SNS. When fully operational, in the 1980's, the SNS will be a world class facility serving a very widely based scientific community. As part of their development activities those appointed will have opportunities to work with research scientists using instruments at Harwell and the Institut Laue-Langevin, Grenoble. In addition, close collaboration is maintained with laboratories in the USA and elsewhere where spallation sources are being built. Applications should have HNC or HND and relevant experience or degree in physics or applied physics. Some experience in fields such as electronics, high vacuum, or cryogenics would be an advantage. Appointments will be made to permanent positions in the Science Group at the level of Scientific Officer or Higher Scientific Officer. Salary scales (at present under review).

Scientific Officer £3,037-£4,724.

Higher Scientific Officer £4,388-£5,820.

Please apply to

Jane Griffiths in the Personnel Group

on Abingdon (0235) 21900 Ext. 510 or

write to her quoting Reference No. VN. 818.

Closing date for applications, July 6th 1979.

# RUTHERFORD Science Research Council

Rutherford Laboratory, Chilton, Didcot,  
Oxfordshire OX11 0QX. Tel Abingdon 21900

2265(A)

MANCHESTER POLYTECHNIC  
John Dalton Faculty of Technology

## HEAD OF DEPARTMENT OF BIOLOGICAL SCIENCES (GRADE VI)

Candidates should be well qualified academically and have research/industrial experience.

Qualities of academic leadership and administrative ability are essential.

The Department operates high level courses in B.Sc./B.Sc. Hons. Biological Sciences, H.N.D. Applied Biology, M.I.Biol., H.N.C. and Fellowship in Medical Laboratory Sciences, and Biology in the B.Sc./B.Sc. Hons. Combined Studies degree, and co-operates actively with other Faculties and Departments on a variety of courses.

Salary scale: £9,345 to £10,305.

For further particulars and application form (returnable by July 31, 1979) please send a self-addressed envelope marked "T/477" to the Secretary, Manchester Polytechnic, All Saints, Manchester M15 6BH. 2212(A)

LONDON SCHOOL OF  
HYGIENE AND TROPICAL  
MEDICINE

(University of London)

Keppel Street, WC1 7HT

DEPARTMENT OF MEDICAL  
MICROBIOLOGY

A MEDICAL LABORATORY  
SCIENTIFIC OFFICER

is required to work up to one year on the viruses of African haemorrhagic fevers. Candidates must be graduates or hold HNC/HND or an equivalent qualification and have had experience in electron microscopy. Inclusive salary, depending on experience, rises to a maximum of £4,284 (under review). Pensionable employment. Applications, consisting of full career and education details and naming two referees, should be sent to Secretary (A1) at the School. 2238(A)

LEICESTER POLYTECHNIC  
SCHOOL OF CHEMISTRY

Applications are invited for four posts to study for C.N.A.A. Ph.D. degrees in a small, well motivated and friendly department in which twenty research students are investigating problems in six main areas. These posts are renewable for a maximum of three years.

Post No. 1

POLYMER AND  
ADHESION SCIENCE

The object is to elucidate the mechanisms by which water affects the durability of epoxide-metal adhesive joints. The programme, which is directed towards aerospace applications, will be supervised by Dr John Comyn and Dr Derek Brewis.

Post No. 2

ELECTROCHEMICAL  
OXIDATIONS OF  
ORGANIC COMPOUNDS

It is hoped to replace such oxidising agents as permanganate and dichromate, which cause severe effluent problems in the pharmaceutical and other industries, by electrochemical grown metal oxide films and porous electrodes. The programme is supervised by Dr Robert Latham and Dr Ralf Dahm.

Post No. 3

SOLID STATE  
ELECTROCHEMISTRY

The aim is to study fundamental aspects, especially of the interface within the cell, of certain silver-copper and other novel battery systems. The programme is supervised by Dr Roger Linford.

Post No. 4

SOLID STATE  
BATTERY SYSTEMS

The project involves the study of solid state battery systems based on novel ionic conductors. The programme is supervised by Dr Roger Linford.

Salary for posts 1-3:

£2,673 per annum plus annual increments, including six hours/week demonstrating/teaching. These are L.E.A. Research Assistantships.

Salary for post 4:

This is a studentship provided by Mallory Batteries (U.K.) Ltd., pre-employment value £2,150 per annum tax free.

Application forms and further details from the Staffing Office, Leicester Polytechnic, P.O. Box 14 Leicester LE1 9BH; informal enquiries to Roger Linford, Reader in Chemistry (0533 50181, extn. 2202). 2201(P)

THE ROYAL  
VETERINARY COLLEGE  
University of London  
Division of Preclinical Studies  
DEPARTMENT OF PHYSIOLOGICAL  
POSTDOCTORAL  
RESEARCH ASSISTANT

Applications are invited for the above post which is expected to last for three years supported by grant from the Medical Research Council for a project on the interaction between general anaesthetics and neurotransmitters in the spinal cord under the direction of Dr David Lodge.

Applicants should have experience in micro-electrode recording and micro-iontophoretic techniques. They should contact Professor M. G. J. Jukes, Head of Department, or (after July 12) Dr David Lodge, Senior Lecturer, as soon as possible.

Salary £4,858 to £5,342 per annum including London Allowance according to qualifications and experience. Superannuation under the University scheme.

Application form from the Assistant Secretary (Personnel), The Royal Veterinary College, Royal College Street, London NW1 0TU. Telephone 01-387 2898. 2194(A)

## Wyeth Institute of Medical Research

### Gastroenterology

We are seeking to appoint a graduate physiologist or pharmacologist with two or more years experience of research on drugs which affect gastric secretion.

This post could be suitable for someone completing a second degree. The successful applicant will join an existing team which is committed to the discovery and development of new treatments for peptic ulcers. We are looking for someone who will be able to make an immediate contribution to the work of the section.

Please apply to:

Mrs. J. Andrews, Personnel Officer,  
**Wyeth Laboratories,**  
Huntercombe Lane South, Taplow,  
Maidenhead, Berks. Tel. Slough 28311.



## Wyeth Laboratories

2258(A)

### UNIVERSITY OF MAINE AT ORONO DEPARTMENT OF ZOOLOGY ASSISTANT PROFESSOR VERTEBRATE ECOLOGIST

Position to begin September 1, 1979. Salary \$13,000. Ph.D. required. Anticipated teaching responsibilities: part of undergraduate vertebrate biology course, alternate year avian biology and mammalogy, and graduate level community ecology. Conduct research in community ecology, with emphasis on birds and/or mammals.

Send credentials, reprints, and three letters of reference to: Dr Franklin L. Roberts, Chairman, Department of Zoology, University of Maine, Orono, Maine 04469. Deadline for receipt of applications May 20, 1979.

An Affirmative Action/Equal Opportunity Employer. W178(A)

### UNIVERSITY OF HAMBURG INSTITUT FÜR PHYSIOLOGISCHE CHEMIE ABTEILUNG ZELLBIOCHEMIE Applications are invited for the post of POSTDOCTORAL RESEARCH FELLOW

The post which is financed by the Deutsche Forschungsgemeinschaft is for work on biosynthesis of specific peptides and proteins from brain and particularly suitable for someone with experience in peptide analysis (sequencing and synthesis). Salary approximated DM37,000. Applications, together with the names and addresses of two referees should be forwarded to Prof. Dr D. Richter, Universität Hamburg, Institut Physiologische Chemie, 2 Hamburg Martinistr. 52, Pav. 53, Germany. W180(A)

### ODENSE UNIVERSITY DENMARK

A new position as Professor in Photosynthesis has been created in the Institute of Biochemistry, and is available for occupation as soon as possible.

It is desired to appoint a person whose research activities are experimental investigations of the functions of the photosynthetic membrane systems in eukaryotes and/or prokaryotes. A part of the current research in the Institute is concerned with electron transport in respiratory and photosynthetic systems, and the Institute is well equipped with special apparatus for this type of work.

The successful applicant will be expected to participate in the teaching programme of the Institute, which includes elementary courses common to science and medical students, advanced courses for science students and supervision of advanced project work.

Further information is obtainable from Institute of Biochemistry, Odense University, telephone (09) 15 86 00, ext. 2441.

The application should contain information about the candidate's teaching experience. It is expected that the successful applicant will eventually be able to teach in Danish.

The employment field covers the Ministry of Education and the institutions under it. The wage frame is 37, and the salary amounts to Danish Kroner 230,520.24 a year inclusive of bonuses as per the October 1, 1978.

A professional selection committee will discuss the applications and their recommendation will be sent out to all applicants in its complete form.

Application in 5 copies, marked "Position No. 745" enclosing curriculum vitae and documentation for professional and pedagogical activities must be made to the Queen and sent together with all enclosures also in 5 copies to: Journalkontoret, Odense University, Campusvej 55, DK 5230 Odense M, Denmark, so that we will receive it by October 1, 1979 at the latest. W173(A)

# SCHERING erwartet Sie

Für das Department Entzündungspharmakologie  
unserer Pharma Forschung suchen wir einen

## Mediziner/Medizinerin oder Biologen/Biologin

als Leiter einer wissenschaftlichen Arbeitsgruppe.

Wir gehen davon aus, daß sich aus den Erkenntnissen der immunbiologischen Grundlagenforschung Impulse zur Therapie bisher unzulänglich behandelbarer Erkrankungen ergeben werden.

Wir wünschen uns einen Mitarbeiter, der aufgrund seiner mehrjährigen experimentellen Erfahrung in der Lage ist, sich an dieser Grundlagenforschung zu beteiligen. Der Schwerpunkt seiner wissenschaftlichen Arbeiten wird auf dem Gebiet der Immunologie und Molekularbiologie liegen; dort wird die Basis für eine spätere Etablierung von Modellen zur Wirkstofffindung gelegt.

Wenn Sie an dieser Aufgabenstellung interessiert sind, bewerben Sie sich bitte schriftlich mit den üblichen Unterlagen oder rufen Sie uns an (Tel.: 030/468 28 53 oder 468 29 22).

SCHERING AKTIENGESellschaft  
Personalabteilung Berlin  
Müllerstraße 170-178, 1000 Berlin 65

# SCHERING AG

W172(A)

### CHARING CROSS HOSPITAL MEDICAL SCHOOL (University of London) GRADUATE RESEARCH ASSISTANT

(Biochemistry/Cell Biology)

Applications are invited for the above post in the Department of Experimental Pathology to assist in a two-year project on collagen synthesis in experimental renal fibrosis. The starting salary will be £3,689 plus £502 London Weighting. Further details may be obtained from Dr M. Barrett, telephone 01-748 2040 ext. 2761.

Applications on forms obtainable from The Secretary, Charing Cross Hospital Medical School, The Reynolds Building, St. Dunstan's Road, London, W6 8RP to be submitted by July 6, 1979. (Ref: 013/4). 2229(A)

### HERPETOLOGIST HARVARD UNIVERSITY

seeks to make a tenure-track appointment in herpetology of an assistant or associate professor in The Department of Biology who will serve conjointly as an assistant or associate curator in The Museum of Comparative Zoology. Preference will be given to candidates with (1) a strong research program in herpetology, (2) ability to offer instruction at the undergraduate and graduate levels, and (3) commitment to supervise and enhance a major herpetological collection. The closing date for receipt of applications is October 15, 1979, and the appointment will take effect July 1, 1980. Applicants should send a curriculum vitae, statement of research interests, and names of three references to: Herpetology Search Committee, Office of the Chairman, Department of Biology, Harvard University, Cambridge, Massachusetts 02138. Harvard is an Equal Opportunity/Affirmative Action Employer. W176(A)

# CSIRO AUSTRALIA

## Postdoctoral Research Fellow Division of Horticultural Research Adelaide

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees 2,400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia. FIELD: Salinity Research.

GENERAL: The Division is concerned with research on a range of perennial horticultural plants (deciduous and evergreen) from both temperate and subtropical regions. Current programmes include studies on the management and genetic improvement of grapevines and fruit trees and of plant viruses and plant parasitic nematodes. Research in plant physiology, biochemistry and cytology relates to photosynthesis, salinity and plant and fruit development.

LOCATION: The Division has laboratories at Adelaide, South Australia (Headquarters); and Merbein, Victoria. The appointee will be located at the Merbein laboratory.

DUTIES: To develop and select salt tolerant crop plants in collaboration with plant physiologists. Experimentation would involve annuals as well as perennial horticulture species.

QUALIFICATIONS: A Ph.D. degree in plant breeding and genetics, or equivalent qualifications.

SALARY: Research Scientists or Senior Research Scientist: \$A14829-\$A21543 pa.

TENURE: 3 years. Superannuation available.

Applications in DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 491/052 should reach:—

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 20th July 1979.

Applications in U.S.A. and Canada should be sent to:—The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, N.W., Washington, D.C., 20036, U.S.A. 2243(A)

## THE MIDDLESEX HOSPITAL MEDICAL SCHOOL

(University of London)

SUB-DEPARTMENT OF

MOLECULAR BIOPHYSICS

DEPARTMENT OF

NUCLEAR MEDICINE

W.H.O. Collaborating Centre

for

Research and Reference Services

in the

Immunoassay of Hormones

in Human Reproduction

Applications are invited from

**BIOCHEMISTS**

experienced in radioimmunoassay and related techniques for a vacant post in the Supra-regional Assay Service Laboratory. The successful applicant will be primarily responsible, in collaboration with a senior endocrinologist, for the day-to-day running of the routine radioimmunoassay laboratory. He/she will, in addition, play a principal role in the development of new immunoassay techniques and the application of new and conventional methodologies for the assay of hormones and other substances of biological importance.

The appointment will be governed by University Conditions and Terms of Service, with starting salary at an appropriate point on the Lecturer Scale, £4,761 to £8,424 per annum, inclusive of £502 per annum London Allowance.

Applications, including a curriculum vitae and the names and addresses of two referees, to Professor R. P. Ekins, Department of Nuclear Medicine, The Middlesex Hospital Medical School, WIN 8AA. 2195(A)

## UNIVERSITY OF LIVERPOOL

Department of Organic Chemistry

**TECHNICIAN**

to assist with work concerning fungal biosynthesis. A major interest in microbiological techniques is preferable and knowledge of chemistry advantageous. Candidates must possess O.N.C. or equivalent as minimum qualification, but applications from candidates holding H.N.C. or degree with some previous laboratory experience will be welcomed.

Initial salary within a range up to £3,708 per annum according to qualifications and experience. Application forms available from the Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote Ref: RV/654/N. 2224(A)

## THE UNIVERSITY OF LIVERPOOL

Selwyn Lloyd Chair of  
Medical Entomology

Applications are invited for the Selwyn Lloyd Chair of Medical Entomology, a full-time Chair held in the Liverpool School of Tropical Medicine. The appointment will be made as a non-clinical appointment. The salary will be not less than £11,484 per annum.

Applications (15 copies), together with the names of three referees, should be received not later than July 26, 1979 by the undersigned from whom further particulars may be obtained. (Candidates overseas may send only one copy by airmail.) Quote Ref RV/659/N. H. H. Burchall Registrar, The University P.O. Box 147, Liverpool, L69 3BX. 2225(A)

## THE POLYTECHNIC OF WALES Politechnig Cymru

Applications are invited for the following post:

### LECTURER II/ SENIOR LECTURER

DEPARTMENT OF SCIENCE

The person appointed, who will be a Microbial Physiologist/Microbial Biochemist will be required to teach up to B.Sc. (Honours) level and should have a higher degree together with relevant research, teaching or industrial experience. In addition he/she will be expected to undertake research.

Salary £4,101 to £7,065 (bar) to £7,572 (subject to review with effect from April 1, 1979).

Application forms and further particulars available from:

The Personnel Officer,  
The Polytechnic of Wales,  
Pontypridd,  
Mid Glamorgan,  
CF37 1DL.

Closing date: July 9, 1979.

2202(A)

## THE POLYTECHNIC OF CENTRAL LONDON BIOMEDICAL RESEARCH GROUP RESEARCH ASSISTANT

Salary £3,087 to £3,261 inclusive of London Allowance

A vacancy exists for a Research Assistant (3 year appointment) to work on brain evoked responses to sensory stimuli in neonates. The project will be carried out jointly with University College Hospital, London. The successful candidate, if suitably qualified, will be able to register for a higher degree (M.Phil. or Ph.D.).

Closing date: Two weeks after appearance of advertisement.

Application form and further details from the Establishment Officer, PCL, 309 Regent Street, London W1R 8AL (Tel. 01-580 2020, ext. 212).

2207(A)

## UNIVERSITY OF MALAYA

Applications are invited for the following Chairs:

Faculty of Science

**CHAIR OF GEOLOGY**

Faculty of Engineering

**CHAIR OF BIOCHEMICAL**

**ENGINEERING**

Computer Centre

**Qualifications and Experience:** Candidates should possess: a Ph.D. in the required field with:—(a) three years' experience as Senior Lecturer/Reader/Associate Professor; or (b) five years' experience as Lecturer; or a Master's degree in the required field with:—(a) five years' experience as Senior Lecturer/Reader/Associate Professor; or (b) eight years' experience as Lecturer.

Candidates are also required to undertake research and to have publications of academic standing.

**Salary Scales** (All inclusive [approx. stg. equiv.]: £8,313 by £420 to £8,733/Review Point/£9,014 by £420 to £9,434 per annum.

Further particulars and application forms are obtainable from the Association of Commonwealth Universities (Acpus), 36 Gordon Square, London WC1H 0PF.

The closing date for the receipt of applications is July 20, 1979.

2217(A)

## THE UNIVERSITY OF TORONTO ASSISTANT PROFESSOR IN BIOCHEMISTRY

Applications are invited for positions of Assistant Professor in Department of Biochemistry (or be located in the Playfair Unit in the Department of Biochemistry) to take effect July 1, 1980. Both appointees will be on a two-year contract with possibility of renewal for a further three years. The successful applicants will be required to take part in the tea programs of the Department and develop a research program. The appointment to the Playfair Unit will be made in the area of membrane receptor biochemistry; the other position has no field restrictions. Department is involved in tea Biochemistry to undergraduates in Faculties of Medicine, Dentistry, and Science, and Nursing, as well as graduate students. The minimum salary for both positions will be \$18,000.00.

Applicants with suitable qualifications should send a curriculum vitae and the names and addresses of referees to: Chairman of the Selection Committee, Department of Chemistry, University of Toronto, Canada, M5S 1A8, to him not later than September 1979. W182

## UNIVERSITY OF NEBRASKA-LINCOLN DEPARTMENT OF PLANT PATHOLOGY RESEARCH ASSOCIATE

Two Postdoctoral Fellows for temporary positions (2 years) available September 1979.

(1) Methodology involves separation of proteins by gel electrophoresis identifying Fe proteins by autoradiography.

(2) Study transmission of mosaic virus by the Mexican beetle. Transmission of electrophoretic forms will be compared and far radio labelled virus will be studied.

Salary about \$13,000/year. Send application or request details from L. C. Lane, Plant Pathology Department, The University of Nebraska-Lincoln, Lincoln, Nebraska, (402-472-3165) Affirmative Action Equal Opportunity Employer. W185

## UNIVERSITY OF SYDNEY LECTURESHIP IN ANIMAL HUSBANDRY (Animal Genetics)

Candidates should be graduates in Veterinary Science, Agriculture, Science, Rural Science or equivalent with a higher degree in field quantitative genetics, animal breeding or population genetics. Appointee responsible to Head of Department of Animal Husbandry for courses in Veterinary Science, Agriculture. He/she will also be expected to participate in the programme of research and postgraduate training in quantitative animal breeding and population genetics conducted within the Department.

The position is expected to be held by a probationary appointee for three years, capable of leading tenure, but if all the University requirements for tenure are met, tenure may be granted at the time of appointment.

Salary range: \$A15,786 to \$A2 per annum.

Applications, including curriculum vitae, list of publications and names of three referees, by August 31, to the Registrar, University of Sydney NSW 2006, Australia, from whom further information available. Information also available from Association of Commonwealth Universities (Acpus), 36 Gordon Square, London WC1H 0PF. 21861



# UNIVERSITY OF SYDNEY LECTURESHIP IN MATHEMATICAL STATISTICS

Preference given to applicants interested in one or more of: Statistical Inference, Robustness, Time Series, Statistical Computations, and in applications to analysis of data. Teaching capability will be an important consideration.

The position is expected to be filled by a probationary appointment of three years, capable of leading to tenure, but, if all the University's requirements are deemed to be satisfactorily met tenure may be granted at the time of appointment.

Salary range: \$A15,786 to \$A20,737 per annum.

Applications, including curriculum vitae, list of publications and names of three referees by July 31, 1979 to the Registrar, University of Sydney, NSW 2006, Australia, from whom further information available. Information also available from Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. 2187(A)

# UNIVERSITY OF HONG KONG CLINICAL BACTERIOLOGIST

Applications are invited for a post of Clinical Bacteriologist in the Department of Microbiology. Applicants with a medical qualification and suitable laboratory experience are preferred though a science graduate with experience in hospital microbiology service will also be considered.

Annual salaries (superannuable) are: Medically qualified: HK\$63,900-71,140 - 74,100 - 81,540 - 85,320 BAR 02,780 - 107,340 - 111,960 - 116,580 - 121,200.

Non-medically qualified: HK\$46,260 - 43,200 - 63,540 x 4,260 - 72,060 BAR 6,320 x 4,260 - 106,140. (£1 equals HK\$10.30 approx.).

Starting salary will depend on qualifications and experience.

At current rates, salaries tax will not exceed 15 per cent of gross income. Housing at a rental of 7½ per cent of salary, education allowances, long leave and medical benefits are provided.

Further particulars and application forms may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or the Assistant Secretary (Recruitment), University of Hong Kong, Hong Kong. Closing date for applications is July 1, 1979. 2192(A)

# DEVELOPMENTAL BIOLOGISTS

The Department of Biology of The Johns Hopkins University has 2 faculty positions available. One of these positions is at the assistant professor level while the other may be any level. Individuals interested in research and teaching in the cellular, genetic and/or molecular aspects of developmental biology are invited to apply.

Applications will be accepted until September 30, 1979. Send curriculum vitae, bibliography and a description of research interests to:

Chairman, Developmental Biology Search Committee  
Department of Biology  
The Johns Hopkins University  
Baltimore, Maryland 21218

The Johns Hopkins University is an Equal Opportunity Employer and does not discriminate on the basis of race, colour, religion, national origin, age, sex, veteran status, handicap, or any other occupationally irrelevant criteria. W183(A)

# WELSH NATIONAL SCHOOL OF MEDICINE (University of Wales) TENOVUS INSTITUTE FOR CANCER RESEARCH

Applications are invited for the post of

## SENIOR LECTURER

in Cancer Research (Steroid Biochemistry) in the above Institute at Heath Park, Cardiff. Candidates must have considerable research experience in steroid biochemistry and expertise in surgical procedures would be advantageous.

Salary on the scale for Non-clinical Senior Lecturers £8,182 to £10,097 per annum (under review); starting point dependent upon qualifications and experience.

Further particulars available from the Registrar and Secretary, Welsh National School of Medicine, Heath Park, Cardiff, to whom applications should be submitted one month from the appearance of this advertisement. 2214(A)

# UNIVERSITY OF BRISTOL DEPARTMENT OF BOTANY

Applications are invited for the post of

## N.E.R.C. RESEARCH ASSISTANT

in Palaeobotany to work on a monograph of Devonian megaspores under the supervision of Dr K. C. Allen. This appointment will be for three years. Applicants should hold, or expect to obtain, a good Honours Degree in either Botany or Geology. The starting salary will be £3,689 per annum (subject to revision on October 1, 1979) and will commence on or around September 1, 1979.

Applications, including a curriculum vitae, giving the names and addresses of two referees should be sent to Miss Suzy Oakes, Department of Botany, The University, Woodland Road, Bristol BS8 1UG not later than July 5, 1979. 2215(A)

# THE UNIVERSITY OF MANCHESTER Oxford Road Manchester M13 9PL GRADE 4 TECHNICIAN

required in the Electron Microscope Unit of the Department of Botany and Zoology. Applicants should have at least O.N.C. or equivalent qualification and preferably H.N.C. and have had six years' relevant experience including ultramicrotomy and other preparatory techniques.

Salary scale: £3,222 to £3,708 per annum.

Applications with full details of age, qualifications and previous experience should be sent as soon as possible to Doctor R. Butler, Department of Botany. 2235(A)

# UNIVERSITY OF LONDON READERSHIP in Probability and Statistics at University College London

The Senate invites applications for the above Readership in the Department of Statistics and Computer Science, particularly from persons with research interests in data analysis. Salary at an agreed point on the scale £8,698 to £10,775 (from October 1979) plus £502 London Allowance. Applications (10 copies) must be received not later than July 13, 1979 by the Academic Registrar (N), University of London, Senate House, London WC1E 7HU, from whom further particulars should first be obtained. 2190(A)

# AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited from suitably qualified persons for appointment to the following positions:

## RESEARCH SCHOOL OF BIOLOGICAL SCIENCES MOLECULAR BIOLOGY UNIT POSTDOCTORAL FELLOW

The Unit (Head, Dr H. Naora) is exploring the molecular mechanisms of post-transcriptional control in animal cells. This includes the problems of the mRNA-mRNA precursor-product relationship, the nuclear processing of mRNA-precursor molecules, using nucleic acid sequencing and DNA cloning. Applicants should have experience in this particular field of research.

CLOSING DATE: July 31, 1979.

## RESEARCH SCHOOL OF EARTH SCIENCES RESEARCH FELLOW IN GEOPHYSICAL FLUID DYNAMICS

The School wishes to make an early appointment to a third Research Fellowship in the Geophysical Fluid Dynamics Group. The Group comprises at present Professor J. S. Turner and two Research Fellows.

The research of the Group has been in two main areas: (i) laboratory and theoretical models of convection and mixing processes in the ocean and (ii) theoretical work on fluid dynamical processes in the interior of the Earth. The Group has also developed interests in fluid processes relevant to the formation of ore deposits and of layered igneous intrusions, and would consider further collaborative extensions of research into relevant fields of environmental geochemistry.

Applications are invited from candidates with qualifications in any of these fields. There will be excellent opportunities for the successful candidate to pursue interdisciplinary research with other groups in the School.

Applications should be sent as soon as possible.

## RESEARCH SCHOOL OF PHYSICAL SCIENCES MOUNT STROMLO AND SIDING SPRING OBSERVATORIES FELLOW (One position)

## RESEARCH FELLOW/SENIOR RESEARCH FELLOW (Two positions)

The Observatories expect to make one tenured appointment (Fellow) and one or two short term appointments (3 to 5 years as Research Fellow/Senior Research Fellow). Applications in all fields of observational and theoretical astrophysics will be considered. Astronomers interested in space research applications are encouraged to apply. Applications from senior astronomers who could accept an appointment for one or two years only are welcomed.

CLOSING DATE: August 31, 1979.

### Conditions of Appointment

Salaries: Salary will be in accordance with qualifications and experience within the range—Senior Research Fellow \$A22,049 to \$A26,301; Research Fellow \$A15,786 to \$A20,606; Fellow \$A18,401 to \$A24,652; Postdoctoral Fellow—a fixed point between \$A15,786 to \$A20,606 per annum. Present exchange rates are \$A1=UK£0.53, \$US1.10, \$C1.27.

### Term of Appointment

Fellow—five years in the first instance with the possibility of reappointment after review to age 65. Research Fellow/Senior Research Fellow—three years in the first instance with the possibility of extension to five years. Postdoctoral Fellow—not less than one year or more than two years.

### Other Conditions

Reasonable appointment expenses are paid. Superannuation benefits are available for applicants who are eligible to contribute. Assistance with finding accommodation is provided for an appointee from outside Canberra. The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should obtain the further particulars from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, before submitting applications. 2191(A)

# UNIVERSITY OF ST ANDREWS DEPARTMENT OF ANATOMY AND EXPERIMENTAL PATHOLOGY

Applications are invited for the recently created post of

## LECTURER

in the Department of Anatomy and Experimental Pathology. The successful candidate will be expected to contribute to the teaching of

### CELLULAR PATHOLOGY.

Previous experience in a Department of Pathology or Experimental Pathology is therefore essential. Excellent facilities for research are available. The Department may be visited by arrangement with Professor D. Brynmor Thomas (Telephone St Andrews 72411).

Salary at appropriate point on scale £4,232 to £8,452 per annum (under review), starting salary not above £6,108, plus F.S.S.U./U.S.S.

Applications (two copies preferably in typescript) with the names of three referees should be lodged by July 9, 1979 with the Establishments Officer, The University, College Gate, St Andrews, Fife, from whom further particulars may be obtained. 2228(A)

# UMIST POSTDOCTORAL RESEARCH in IMAGE ANALYSIS

An image analysis unit is being developed within UMIST for the study of morphological and optical characteristics of cellulose fibres and paper sheets. A postdoctoral research position has arisen in this field which will involve the use of a recently acquired Joyce Loeb Magiscan Image Analyser. The position will have a research bias and be of 2 years' duration. The successful applicant is likely to have a background in physics, electrical engineering, mathematics or computing, but other disciplines would be considered. Salary will be in the range £3,883 to £6,555 per annum (which is subject to a revision from October 1978 and a further award from October 1979).

Applications by letter, quoting reference PFS/58/A1 giving curriculum vitae and the names of two referees should be addressed to Dr J. C. Roberts, Paper Science Building, UMIST, PO Box 88, Manchester M60 1QD. 2173(A)

## CSIRO AUSTRALIA

### Postdoctoral Research Fellow

#### Division of Protein Chemistry Parkville Victoria

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees 2,400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

**FIELD:** Immunochemistry.

**GENERAL:** The Division has a research staff of some 60 biochemists, biophysicists, physical biochemists and organic chemists. The major role of the Division is to contribute to knowledge of the molecular structure and chemistry of proteins, particularly in areas where the molecular properties of proteins determine the commercial value of a product or where information is important for plant or animal production. Current research interests include wool, leather, plant proteins and biologically active proteins. Plant protein research is directed to the increased and more efficient utilization of seed protein from legumes in Australian agriculture and industry.

**DUTIES:** Initially, to study the characterization and assay of seed storage proteins using serological techniques and to provide immunological data for comparative studies of proteins from different sources. The person appointed will be encouraged to apply his/her knowledge of immunochemistry to other relevant research programmes within the Division.

**QUALIFICATIONS:** A PhD in an appropriate field with demonstrated research ability. Experience in the field of immunochemistry is essential.

**SALARY:** Research Scientist/Senior Research Scientist: \$A15,422-\$A22,405 pa.

**TENURE:** Fixed term of 3 years.

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 462/448 should reach:—

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 20th July 1979.

Applications in U.S.A. and Canada should be sent to:—

The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, N.W., Washington, D.C., 20036, U.S.A. 2245(A)

## AGRICULTURAL RESEARCH COUNCIL

### FOOD RESEARCH INSTITUTE

#### SCIENTIFIC SERVICES AND DEVELOPMENT DIVISION

### CHEMIST

A Scientific Officer is required to join, on a temporary basis, the Chromatography Development Group which is part of the Scientific Services and Development Division. The appointment relates to a research contract ending in October 1980, which has as its objective the estimation in poultry waste of residues of specified veterinary products. Published gas chromatographic methods will be worked up but some method development will be necessary.

A pass degree or H.N.C. in chemistry, together with a knowledge and practical experience of high performance liquid chromatography and/or gas chromatography, are required.

Salary: on a scale £2,839 to £4,415 (under review).

Five-day working week and flexible working hours scheme operated. Transport facility available mornings and evenings.

Further particulars and application forms from:

The Secretary, Food Research Institute, Colney Lane, Norwich NR4 7UA, quoting Ref. No. 79/9.

Closing date: July 4, 1979.

2260(A)

**To place your  
advertisement in these pages**

**Phone: 01-831-6901**

## NEW ZEALAND FOREST SERVICE

# Scientist

### PRODUCTION FORESTRY DIVISION, FOREST RESEARCH INSTITUTE, ROTORUA

Applications are invited for the above position. Salary up to \$16,685 (N.Z.) per annum depending on qualifications and experience.

A forest soils scientist is required to join a team of 8 scientists and 15 support staff studying tree nutrition, soil fertility and forest site productivity in New Zealand. A major part of the programme involves pinus radiata plantations and includes soil chemistry, soil physics, tree nutrition, nutrient cycling and biomass production.

Qualifications desired: PhD in soil science with supplementary training and experience in forestry, or PhD in forestry with supplementary training and experience in soil science.

Housing available for an initial 2 year period.

Successful applicants will receive assistance with fares and transfer of personal baggage to New Zealand.

Application forms may be obtained from:

**The Chief Migration Officer  
New Zealand House  
Haymarket  
LONDON  
SW1Y 4TQ**

You should quote reference Imm 2/326/6.

Closing date for applications: August 24th 1979.

Further particulars may be obtained from the Director, Production Forestry Division, Forest Research Institute, Private Bag, Rotorua, New Zealand.

2176(A)

## CHARING CROSS HOSPITAL (FULHAM)

### BIOCHEMIST

#### DEPARTMENT OF MEDICAL ONCOLOGY

Applications are invited from Chemists/Biochemists to join a project on synthesis and *in vivo* localisation of antibody conjugates relevant to diagnosis and therapy of cancer. Candidates should possess an appropriate science degree and experience of radioimmunoassay would be an advantage. The post will be supported by the Cancer Research Campaign and Whitley Council salaries and conditions for Probationary Grade Biochemists apply.

Further details from Dr F. Searle, Medical Oncology, tel: 01-748 2040, ext. 2320. Application forms from District Personnel Department, Charing Cross Hospital, Fulham Palace Road, London W6 8RF. Tel: 01-748 2040, ext. 2992.

2257(A)

**TECHNICIAN or JUNIOR TECHNICIAN** required by the Institute of Cancer Research in association with the Royal Marsden Hospital at Sutton, Surrey. Candidates should have an interest in cell culture and, ideally, some previous experience in this field and also in enzymology. The individual appointed will take part in a new M.R.C. Project on clinical and laboratory aspects of head and neck cancer, commencing September-October, 1979. Salary in scale £3,261 to £3,813 p.a. (Technician) or £2,169 to £3,015 p.a. (Junior Technician), plus London Allowance of £354 p.a. (scales under review). Salary will depend on age and qualifications, two 'A' levels in science subjects for Junior Technician and either a Degree, HNC or equivalent qualification for a Technician appointment. Applications in duplicate with the names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place, London SW7 3NU, quoting ref. 301/B/73.

2174(A)

## UNIVERSITY OF LIVERPOOL DEPARTMENT OF ZOOLOGY

Applications are invited for the post of

### POSTDOCTORAL SENIOR RESEARCH ASSISTANT (Grade 1A)

to work on the mechanisms of ion transport in fish erythrocytes and the alterations of ion transport during salinity acclimation of fish. Techniques involved include isotope flux and cell culture.

Initial salary £4,232, £4,505 £4,776 per annum.

Applications, together with names of three referees, should be received not later than July 16, 1979 by The Registrar, The University, Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref. RV/650/N.

2169(A)



REQUIRED BY THE  
FOOD AND AGRICULTURE  
ORGANIZATION OF THE  
UNITED NATIONS, ROME, ITALY

## CROP PROTECTION SPECIALISTS

(Phytopathologists, Entomologists, Weed Scientists)

to be stationed in Sahelian countries, serving in a large-scale project on Integrated Pest Management for Basic Food Crops implemented with the assistance of FAO by the Inter-States Permanent Committee for Drought Control in the Sahel (CILSS).

**ESSENTIALS:** Ph.D. in Plant Protection Sciences. At least 7 years of post-graduate experience in pest management. Good working knowledge of English or French.

**SALARY:** dependent on qualifications/seniority, net tax-free including the usual International Civil Service allowances. Please send detailed curriculum vitae quoting "TF/RAF/128(USA)-comm." to Mr T. Eshetu, Manpower Planning, AGO, FAO, Via delle Terme di Caracalla, 00100 Rome, Italy.

W184(A)

### BIOCHEMIST

Our Department of Biochemistry is seeking an experienced scientist to work in the field of drug metabolism with special reference to the skin.

The successful candidate will have:

- At least five years' postdoctoral experience (or the equivalent).
- *In vivo* and *in vitro* experimental ability in metabolism work, including the handling of labelled compounds. Experience in skin biochemistry would be advantageous.
- A practical knowledge of analytical chemical techniques (HPLC, GC, TLC and spectroscopy).

Our modern Research Centre based near Nice in the South of France, was recently created to study the skin and its disorders and is associated with a multi-national French Company.

For further details please send curriculum vitae to Box No. W181(A), c/o Macmillans Journals, 3 Dyers Buildings, Holborn, London EC1N 2NR.

W181(A)

Please mention

# Nature

when replying to  
these advertisements

### UNIVERSITY OF LIVERPOOL DEPARTMENT OF GENETICS

Applications are invited for the post of

#### POSTGRADUATE RESEARCH ASSISTANT

to work on an N.E.R.C. financed project to investigate genetic determination of abundance and polymorphism in a population of *Drosophila melanogaster*.

The project is financed for three years. Candidates should be graduates with a sound background in ecology, genetics or ecological or population genetics. A suitably qualified candidate may be allowed to register for a higher degree.

Applications, together with the names of three referees, should be received not later than July 12, 1979, by The Registrar, The University PO Box 147, Liverpool L69 3BX. Quote Ref. RV/648/N. 2168(A)

**INSTITUTE OF CANCER RESEARCH: TECHNICIAN or JUNIOR TECHNICIAN** required at the Sutton, Surrey laboratories for work with mammalian cell culture applied to radiobiology. Experience in cell culture methods would be an advantage but is not essential. Salary in scale £3,261 to £4,680 per annum. (Technician) or £2,169 to £3,015 per annum. (Junior Technician), plus London Allowance of £354 per annum (scales under review). Salary will depend on age and qualifications, two 'A' levels in science subjects for Junior Technician and a Degree, HNC or equivalent qualification for a Technician appointment.

Applications in duplicate with the names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place, London, SW7 3NU, quoting ref. 301/B/74. 2222(A)

### ROYAL FREE HOSPITAL SCHOOL OF MEDICINE

(University of London)

Department of Neurological Science  
TECHNICIAN

Histological Technician required to work in EM neuropathology laboratory. Appointment for 3 years in first instance. Salary on Whitley Council Scale according to age and experience.

Further details and application form from the School Secretary, R.F.H.S.M., 8 Hunter Street, London, WC1N 1BP, or tel. 01-837 5385 ext. 54. Closing date for applications July 13, 1979. 2221(A)

**INSTITUTE OF CANCER RESEARCH** in association with the **ROYAL MARSDEN HOSPITAL**, Sutton, Surrey, require **JUNIOR TECHNICIAN** in the Department of Histopathology. Applicants should have a minimum of 5 'O' levels including Maths, English and two science subjects or (preferably) two science 'A' levels or ONC in Medical Laboratory Sciences.

Training will be given in all aspects of histological technique and Day-release will be granted.

Salary in scale £2,037 to £3,015 per annum depending on age and qualifications (scales under review), plus London Allowance of £354 per annum.

Applications in duplicate with the names of two referees to the Secretary, Institute of Cancer Research, 34 Sumner Place, London, SW7 3NU, quoting ref. 301/B/75. 2236(A)

### STUDENTSHIPS

#### UNIVERSITY OF LONDON REACTOR CENTRE ASCOT, BERKSHIRE

##### POSTGRADUATE RESEARCH STUDENTSHIPS

Applications are invited, from candidates holding, or expecting to obtain, a good honours degree, for the following research studentships tenable from October 1:

- (i) A Bursary Funded by the U.K.A.E.A. for a study of the Radioactive Decay Schemes of Actinide Isotopes in collaboration with the Nuclear Physics Division at A.E.R.E. Harwell.
- (ii) An S.R.C. Case Studentship in collaboration with the National Physical Laboratory for a project involving the measurement of Neutron Energy Spectra and Neutron Absorption Cross Sections in the Resonance Region.
- (iii) An N.E.R.C. studentship in collaboration with the British Museum (Natural History) for a study of the structural breakdown of Minerals by Radioactivity and involving the use of particle track mapping techniques.
- (iv) S.R.C. Quota Awards may be available for various other projects in fields such as Reactor Physics, Neutron Activation Analysis and Radiochemistry.

Those interested are invited to write or telephone, as soon as possible, for an application form and further details to:

Dr T. D. MacMahon, University of London Reactor Centre, Silwood Park, Sunninghill, Ascot, Berkshire SL5 7PY. (Telephone Ascot (0990) 23911 ext. 298) 2219(F)

#### IMPERIAL COLLEGE UNIVERSITY OF LONDON

DEPARTMENT OF CHEMICAL ENGINEERING AND  
CHEMICAL TECHNOLOGY

##### S.R.C. C.A.S.E. STUDENTSHIPS

for research with Dr G. C. Maitland into the following areas:

1. Elongational flow of polymer solutions in relation to enhanced oil recovery, in collaboration with B.P. Research Centre;
2. Mathematical modelling of P.V.C. polymerisation processes, in collaboration with I.C.I. Plastics Division.

Applicants should hold, or expect to obtain, a first or upper second class honours degree in a physical science or chemical engineering. The awards are tenable for three years at normal S.R.C. rates supplemented by up to £500 per annum by the sponsors.

All applications, including a curriculum vitae and the names of two referees, should be sent to:

Dr K. E. Bett  
Department of Chemical Engineering  
Imperial College  
London SW7 2BY

2261(F)



## STUDENTSHIPS—continued

# university college of swansea

## RESEARCH STUDENTSHIP

The Science Research Council is prepared this year to offer to suitable candidates a Research Studentship tenable in the Department of Geology or the Department of Oceanography at the above University College.

Applicants should have at least an upper second class honours degree in an appropriate science, or be expected to obtain such a degree by July, 1979, in order to pursue a research programme leading to a Ph.D.

The studentship will be tenable from October 1, 1979 and the value will be in line with current S.R.C. rates.

Further details about the research topics available and application forms may be obtained from the Secretary of the Department of Geology or Oceanography, University College of Swansea, Singleton Park, Swansea SA2 8PP. 2242(F)

## THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF MEDICINE

Man or woman

### BIOLOGY GRADUATE

minimum class II(i), wanted for Medical Research Council Studentship. Those expecting to graduate this summer may apply. A three-year award to study for a Ph.D. in respiratory physiology. Project concerns changes in the lung circulation in oxygen deficiency. Apply to the Registrar and Secretary, The University, Sheffield S10 2TN as soon as possible. Quote Ref. R/305/G. 2170(F)

## UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF  
NEUROPHYSIOLOGY

### RESEARCH STUDENTSHIP IN NEUROPHYSIOLOGY

A Research Studentship is available in Neurophysiology or Neuropharmacology. Applicants should have an Upper Second Class Honours Degree in an appropriate subject. Applications giving names and telephone numbers of two referees should be sent to Professor G. A. Kerkut, Department of Neurophysiology, School of Biochemical and Physiological Sciences, Southampton University, Southampton. 2175(F)

## UNIVERSITY OF NEWCASTLE

Department of Microbiology

### S.R.C. C.A.S.E.

### RESEARCH STUDENTSHIP

Applications are invited for a C.A.S.E. Studentship in co-operation with the Fish Diseases Laboratory, Weymouth to investigate the taxonomy and ecology of the causative agents of Bacterial Kidney Disease in Fish. Applicants should hold or expect to achieve at least an upper second class Honours Degree in Microbiology or have an equivalent qualification.

Applications with the names and addresses of two referees and a curriculum vitae should be sent to Dr M. Goodfellow, Department of Microbiology, The Medical School, The University, Newcastle upon Tyne NE1 7RU by June 30, 1979. 2233(F)

## THE HATFIELD POLYTECHNIC

BIOLOGICAL SCIENCES

### S.R.C. STUDENTSHIP

An S.R.C. Studentship is available in one of the following areas:

- (i) Viricidal effects in plant tissue cultures.
- (ii) Drug metabolism studies with isolated Hepatocytes.
- (iii) The pelleting of Actinomyces in submerged culture.
- (iv) *Microspora alaphoides*, Powdery Mildew on *Quercus robur*.

Applicants must possess a good honours degree in an appropriate subject and will be expected to register for a higher degree. There will be an opportunity to undertake some demonstrating to undergraduates.

For further details apply to Dr K. Wilson, Biological Sciences, The Hatfield Polytechnic, P.O. Box 109, Hatfield, Herts. AL10 9AB, enclosing a curriculum vitae and the names of two referees. 2211(F)

## UMIST

### S.R.C. C.A.S.E.

### STUDENTSHIP

The Science Research Council has made a co-operative award to the Department of Polymer and Fibre Science (Paper Science Section), UMIST, and the Research Association for the Paper and Board, Printing and Packaging Industry (PIRA).

The work will be concerned with investigations into the effects of non-cellulosic polysaccharides on pulp and paper properties. A successful applicant will be expected to submit for a higher degree and will spend some part of his training in the Research Department of PIRA. The maintenance grant is £2,320 per annum, which includes an annual contribution of £500 by PIRA.

Applications are invited from graduates with good honours degrees in Chemistry, Biochemistry or a related subject and should be made in writing, giving a curriculum vitae and the names of two referees, to Dr J. C. Roberts, Paper Science Building, UMIST, PO Box 88, Manchester M60 1QD. 2208(F)

DEPARTMENT OF BIOCHEMISTRY

## UNIVERSITY OF NOTTINGHAM

Queen's Medical Centre  
Nottingham NG7 2UH

### S.R.C. C.A.S.E.

### STUDENTSHIP

A C.A.S.E. studentship is available from October 1, 1979, to work on "Hormonal Regulation of Enzyme Turnover in Adipose Tissue". The project is to be carried out in conjunction with Dr R. G. Vernon, Department of Physiology, The Hannah Institute, Ayr, Scotland. Candidates should be prepared to work in both laboratories.

Candidates should write to Dr R. J. Mayer at the Nottingham address. 2178(F)

## UNIVERSITY OF CAMBRIDGE

### S.R.C. STUDENTSHIP

Applications are invited for the above studentship which is for geochemical or stable isotope geochemical studies of lunar samples and or meteorites.

Application together with names of two referees to Dr C. T. Pillinger, Department of Mineralogy and Petrology, Downing Place, Cambridge CB2 3EW. 2264(F)

## ROYAL HOLLOWAY COLLEGE

(University of London)

Egham Hill, Egham, Surrey

DEPARTMENT OF

BIOCHEMISTRY

POSTGRADUATE

STUDENTSHIP

S.R.C. C.A.S.E. studentship (tenable for three years and leading to Ph.D. degree) to undertake a study of the biochemistry of chill damage of bananas in conjunction with Geest Associates Ltd. The work will involve a variety of biochemical techniques and the student will spend four weeks per annum at Geest Associates in Spalding, Lincs. Candidates who have (or are likely to have) a class 1 or 2 (i) honours degree in: biochemistry; chemistry with a biological subject; botany with chemistry should write to Professor J. B. Pridham. 2246(F)

## THE CITY UNIVERSITY

DEPARTMENT OF

CHEMISTRY

### ENERGY RESEARCH

### S.R.C./C.A.S.E. AWARDS

Applications are invited for studentships for work on the following topics:

- (a)  $H_2$  production using renewable energy sources such as wave, solar, hydroelectricity as well as off-peak nuclear electricity. The work will involve the study of the electronic, crystallographic and electrochemical properties of semiconducting oxides and sulphides, and will be done in collaboration with the Central Electricity Research Laboratory, Leatherhead.

- (b) Mechanism of stress corrosion in North Sea Oil and Gas Pipelines. This study will involve electrochemical and metallurgical study of crack propagation mechanism in stressed pipelines as well as computer simulation of mass transfer effects at the crack tip. The work will be done in collaboration with the British Petroleum Research Centre, Sunbury-on-Thames.

Candidates should have a good honours degree in Chemistry, Materials Science, Chemical Engineering or any other related discipline. The awards will be at the S.R.C. research studentship rate plus industrial allowance.

Applications, together with the names of two referees should be sent to Dr A. C. C. Tseung, Department of Chemistry, The City University, Northampton Square, London EC1V 0HB. 2182(F)

## THE POLYTECHNIC OF NORTH LONDON

DEPARTMENT OF

MATHEMATICS

### S.R.C. C.A.S.E. STUDENTSHIP

### IN THEORETICAL

### AERODYNAMICS

Applications are invited for a collaborative research studentship jointly sponsored by the Science Research Council and Rolls-Royce Ltd. The project involves mathematical modelling of the aerodynamic design of turbomachinery components under mechanical constraints.

Applicants should hold, or expect to obtain this year, a good honours degree in Mathematics, or another suitable subject, and satisfy the usual S.R.C. requirements. The successful applicant will be expected to register for the M.Phil./Ph.D. degree of the C.N.A.A.

During the three years of the project the selected candidate will be based at the Polytechnic, but will, normally, be offered employment by Rolls-Royce Ltd, from October 1979 at full salary commensurate with age and qualifications.

Further details and application forms can be obtained from the Head of the Department of Mathematics, The Polytechnic of North London, Holloway, London N7 8DB. 2181(F)

## UNIVERSITY COLLEGE LONDON

DEPARTMENT OF

GEOLOGY

### C.A.S.E. STUDENTSHIP

Applications are invited for N.E.R.C.-funded C.A.S.E. Studentships to be undertaken in conjunction with the Institute of Geological Science. The successful applicant will study known Rare Earth Element and Lead anomalies in soils and stream sediments of mid-Wales in order to establish the chemical processes responsible for their formation. The project is suitable for persons qualified in either geology or chemistry. Applications should be sent to Dr J. N. McArthur, Department of Geology, University College London, Gower Street, London WC1E 6BT from whom further particulars may be obtained. 2193(F)

## AGRICULTURAL RESEARCH COUNCIL

### STUDENTSHIP

at the WEED RESEARCH

### ORGANIZATION

There is a Postgraduate Studentship available at WRO to enable honours graduates to study for a higher degree.

The project to be researched concerned with the incidence, mechanism and importance of vegetative regeneration in grassland.

For further particulars and a application form write to Secretary Weed Research Organization, Be broke Hill, Yarnton, Oxford OX5 1PF, quoting 16/79. Closing date June 30, 1979. 2218(F)

## UNIVERSITY OF LONDON KING'S COLLEGE

### S.R.C. C.A.S.E. STUDENTSHIP

Applications are invited from British graduates holding a First or Upper Second Class Honours degree in Biochemistry or Pharmacology, or from British students expecting to obtain such a degree this summer, to pursue research on tachyphylaxis in human blood platelets and its relevance to the control of platelet responsiveness. The studentship is held in association with CIBA-Geigy (U.K.) Ltd.

Applications should be sent, as soon as possible to Professor M. C. Scrutton, Department of Biochemistry, King's College, Strand, London WC2R 2L. 2263(F)

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## FELLOWSHIPS

UNIVERSITY OF  
ABERDEENRESEARCH FELLOWSHIP OR  
RESEARCH ASSISTANTSHIP  
IN BIOCHEMISTRY

Applications are invited for a Postdoctoral Research Fellowship with Dr J. E. Fothergill's research group, to determine the structure of the serine proteinase chain of CIs, the enzymically active part of the first component of complement.

The appointment for up to 3 years is supported by the MRC; starting date by arrangement. Salary within Range 1A, £4,232 to £5,321 (under review) with appropriate placing.

Predocutorial appointments with suitable interests and experience will also be considered as Research Assistants (salary as above but within Range 1B).

Further particulars from The Secretary, The University, Aberdeen, with whom applications should be lodged as soon as possible but not later than September 1, 1979.

2204(E)

## ROTHMANS FELLOWSHIPS

Applications are invited for Rothmans Fellowships, which are awarded under the Rothmans University Endowment Fund set up by Rothmans of Pall Mall (Australia) Limited to enable Fellows to undertake postgraduate work within an Australian University.

Rothmans Fellowships are of an annual value of up to \$A14,000. A Fellow may be paid travelling expenses incurred in taking up the Fellowship and returning home.

In addition, an amount of \$A1,500 per annum towards fees and expenses including the purchase and maintenance of equipment may be paid to the University where the Fellow is working.

A Fellow shall take up a Fellowship before attaining the age of twenty-eight. The Fellowships are open to graduates of any University who have had at least three years postgraduate experience in research. The Fellowships are not open to permanent members of academic staff or applicants proceeding on sabbatical, study or other leave (including leave without pay). The Fellowships must be held at an Australian University.

Application forms and further details may be obtained from the Secretary, Rothmans University Endowment Fund, C/- The University of Sydney, NSW 2006, Australia. Applications close on September 28, 1979. Information also available from Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF. 2188(E)

UNIVERSITY OF DUBLIN  
Trinity College

A Postdoctoral Fellowship, supported by the National Board for Science and Technology, is offered for two years from October 1, 1979. The project, on ion-containing polymers, is jointly directed by J. V. McBrierty and J. M. D. Coey. It exploits the NMR and Mossbauer techniques to provide molecular information on this rapidly developing class of polymeric materials. Direct experience in some branch of magnetic resonance on Mossbauer spectroscopy would be an advantage.

Salary will be in the range £3,837 to £4,476.

Interested persons should, in the first instance, telephone the Staff Office, Trinity College, on Dublin 772941, Ext. 1678 or Ext. 1775.

The closing date for receipt of completed applications will be July 15, 1979. 2241(E)

RESEARCH FELLOWSHIP  
IMPERIAL CANCER  
RESEARCH FUND

We have a vacancy for a postdoctoral Research Fellow to join a group working on ageing and neoplastic transformation in epithelial cell systems *in vitro*. Special experience in cell culture techniques, cell hybridisation, etc., an advantage.

Appointment will be for three years. Salary according to qualifications and experience.

Further information from Dr L. M. Franks (01-242 0200, ext. 211). Applications with curriculum vitae and names of two referees should be sent to The Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX. 2250(E)

## UNIVERSITY OF BRISTOL

VETERINARY  
RESEARCH FELLOWSHIP

Applications are invited from veterinary graduates for a Research Fellowship to investigate the physiology of cervical dilatation in the ewe. The successful applicant will join a research group in the Department of Anatomy which is currently working on problems of the myometrium and the physiology of relaxin. The group has accommodation for sheep at Bristol and at the School of Veterinary Science, Langford. It is intended that the applicant will employ a variety of research techniques in the elucidation of this problem, including chronic recording of cervical softening *in vivo*. The opportunity exists to investigate the histochemistry of the glycosaminoglycans of the cervix. In addition, the Department offers a wide spectrum of facilities and expertise in electron-microscopy, electro-physiology, polypeptide biochemistry, neurophysiology, hard tissue studies, and experimental surgery.

The Fellowship is provided by the Agricultural Research Council and awards a salary of £4,232 to £8,452 depending upon age and experience. The appointee will be expected to register for a higher degree.

Applications in duplicate which should include a full curriculum vitae together with the names and addresses of two referees should be sent to Professor D. G. Porter, Pre-Clinical Veterinary Studies, Department of Anatomy, The Medical School, University of Bristol, Bristol BS8 1TD. 2248(E)

IMPERIAL CANCER  
RESEARCH FUND  
RESEARCH FELLOWSHIP

A Postdoctoral Biochemist or Molecular biologist with experience in DNA sequencing techniques is required to assist in cloning and to sequence biologically significant regions of higher eukaryotic DNA.

The appointment will be for three years. Salary range £5,823 to £7,129 inc. L.A.

For further information telephone Dr M. Fried (01-242 0200, ext. 297). Applications with curriculum vitae and the names of two referees should be sent to the Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2, quoting Reference 302/79, by July 30, 1979. 2251(E)

UNIVERSITY OF DUNDEE  
POSTDOCTORAL  
BIOCHEMIST

Applications are invited for a S.R.C. grant-financed postdoctoral fellowship to work in collaboration with Dr D. G. Nicholls on a project whose aim is to establish the molecular mechanism by which noradrenaline regulates the exothermic metabolism of isolated brown adipose cells. For reviews see Biochem. Soc. Trans. 5 (1977) 908; New Scientist, April 13, 1978. The Neurochemistry Laboratory is located in the Department of Psychiatry, and has close ties with the Department of Biochemistry.

The post is available from October 1, 1979, but later starting dates would be considered. The appointment is for up to two years.

Salary within the range £4,232 to £4,776.

Informal enquiries may be made to Dr D. G. Nicholls, Neurochemistry Laboratory, Department of Psychiatry, Ninewells Medical School, University of Dundee. Applications, quoting reference EST/61/79J and containing the names of two referees should be lodged with The Secretary, The University, Dundee DD1 4HN as soon as possible. 2197(E)

UNIVERSITY OF SUSSEX  
SCHOOL OF  
BIOLOGICAL SCIENCES  
POSTDOCTORAL  
RESEARCH FELLOWSHIP  
MOLLUSCAN  
NEUROBIOLOGY

To work on an M.R.C.-financed project investigating the regeneration of specific neural connections in the snail brain. Experience in intracellular recording required, together with an interest in developmental neurobiology. Appointment will be for 18 months in the first instance, with extension dependent on the availability of further finance.

Salary within range £4,232 to £6,627 per annum on the Research Fellow grade 1A scale (under review). Applications, detailing c.v. and the names of two referees, as soon as possible to Dr P. R. Benjamin, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG. 2183(E)

THE UNIVERSITY  
OF LEEDSDEPARTMENT OF METALLURGY  
RESEARCH FELLOWSHIP IN  
ELECTRON MICROSCOPY

Applications from suitably qualified candidates are invited for a temporary post of postdoctoral Research Fellow in the above department to assist in the application of high-resolution electron microscopy and microanalysis to a variety of problems in Metallurgy and Materials Science. A JEOL J20CX TEMSCAN system will be used. Applicants should have considerable experience in electron microscopy, ideally but not necessarily in the use of lattice-imaging techniques, STEM or electron probe microanalysis. The appointment will be made for a fixed period terminating on June 30, 1982.

Starting salary in the range £4,333 to £5,777 on the 1A scale for Research and Analogous Staff (£4,333 to £7,521). The scale quoted is effective from October 1, 1979 and is subject to review.

Applications, with curriculum vitae and the names of two referees, should be sent to the Registrar, The University of Leeds, Leeds LS2 9JT (from whom further particulars may be obtained) not later than July 10, 1979. Please quote reference number 70/4/D. 2171(E)

MASSEY UNIVERSITY  
Palmerston North, New Zealand  
DEPARTMENT OF  
MICROBIOLOGY  
AND GENETICS  
POSTDOCTORAL  
RESEARCH FELLOWSHIP

Applications are invited for a postdoctoral fellowship to study DNA homology among legume root nodule bacteria and its relationship to plant specificity. Applicants should have a background in microbiology or biochemistry. Experience in microbial genetics and nucleic acid biochemistry would be an advantage.

The post is available for three years at an initial salary of NZ\$8,500 per annum. Applicants should indicate the earliest date on which they could be available.

Further details of the position can be obtained from Dr B. D. W. Jarvis, Department of Microbiology and Genetics, Massey University.

Details regarding conditions of appointment and of the University may be obtained from the Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF, or the Registrar of the University.

Applications close July 13, 1979. 2189(E)

WEIZMANN INSTITUTE OF  
SCIENCE FELLOWSHIP

For Researchers with Ph.D. and training in plant physiology, biochemistry and cell biology to participate in a project on aspects of selection for pesticide resistance and pesticide metabolism and physiology in cell cultures. One year with possibility of renewal up to three years.

Send curriculum vitae and names of two referees with request for further information to:

Dr J. Gressel,  
Plant Genetics Department,  
Weizmann Institute of Science,  
Rehovot,  
Israel. W177(A)

## GRANTS &amp; SCHOLARSHIPS

UNIVERSITY OF SYDNEY  
THE THOMAS LAWRENCE  
PAWLETT SCHOLARSHIP

Applications are invited from graduates of universities outside Australia who propose to undertake postgraduate study in the Faculty of Agriculture. The scholarship, which is valued at \$A4,200 per annum, is tenable for one year, but may be renewed.

Further information and application forms may be obtained from the Registrar, University of Sydney, NSW 2006, Australia, with whom applications close on July 31, 1979. 2185(H)

THE ROYAL SOCIETY  
GOVERNMENT GRANT  
for Scientific Investigations

Application for grants from the second allotment of the Government Grant for Scientific Investigations for the year 1979 should be made not later than July 15, 1979 on forms of application to be obtained from the Executive Secretary of the Royal Society, 6 Carlton House Terrace, London SW1Y 5AG.

Applicants must be British subjects domiciled in the United Kingdom. Grants may be made to promote and support research in science and to assist scientific expeditions and collections; but not for personal maintenance, payment of stipends or to aid scientific publications. 2179(H)

## GRANTS & SCHOLARSHIPS —continued—

### UNIVERSITY OF SUSSEX

#### MARGARET GRANT MEMORIAL SCHOLARSHIP

Applications are invited from young scientists for the first annual award of this fellowship established to promote the communication of ideas and techniques among cell biologists, biochemists and microbiologists from all countries. Preference will be given to applicants at an early stage of her/his career. The award is to support visits to other laboratories to learn techniques or extend collaborative experiments. Awards will be in the region of £150 to £300 to help with travel and living expenses. Apply in detail to Professor S. Shall, Biochemistry Department, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG. Deadline: August 1, 1979. 2184(HH).

## ASSOCIATESHIPS

### UNIVERSITY OF BIRMINGHAM DEPARTMENT OF BIOCHEMISTRY A RESEARCH ASSOCIATESHIP

is available to assist in the production of monoclonal antibodies to muscle proteins for the study of the origins and diagnosis of muscle disease (supported by a grant from the Muscular Dystrophy Group of Great Britain to Professor S. V. Perry). Salary on the scale £3,775 to £4,333 (plus U.S.S.). (Ref. BS1).

#### A RESEARCH ASSOCIATESHIP

is being funded by the University of Birmingham to develop facilities for the culture of hepatocytes and the monoclonal production of antibodies to enzymes. Salary on the scale £3,775 to £5,488 (plus U.S.S.). (Ref. BS2).

Both posts are tenable from October 1, 1979. Postgraduate experience in tissue culture techniques would be an advantage for both posts. The Muscular Dystrophy funded post is available initially for one year but with the possibility of an extension for a further two years depending on availability of funds. The university funded post is for three years.

Further details may be obtained from Miss L. R. Hayes, Senate Registry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, to whom applications (two copies) should be returned not later than Friday, July 6, 1979. Please quote reference number of post in any enquiry. 2209(O)

### UNIVERSITY OF BIRMINGHAM DEPARTMENT OF BIOCHEMISTRY A RESEARCH ASSOCIATESHIP

is available from October 1, 1979, to evaluate the application of high resolution two dimensional electrophoresis of the protein and other components of human body fluids and tissues in the diagnosis and study of the origins of neuromuscular diseases.

The appointment is supported by a grant from the Muscular Dystrophy Group of Great Britain to Professor S. V. Perry, and is initially for one year but with the possibility of an extension for a further two years depending on the availability of funds.

Salary in the range £3,775 to £4,333 (plus U.S.S.) depending on age and qualifications.

Further details may be obtained from Miss L. R. Hayes, Senate Registry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, to whom applications (two copies) should be returned not later than Friday, July 6, 1979. 2210(O)

## AWARDS

### UNIVERSITY OF LEICESTER DEPARTMENT OF CHEMISTRY S.R.C. C.A.S.E. AWARDS

Applications are invited from graduates (or potential graduates) with a degree with First or Second Class Honours (Upper Division) in Chemistry, or equivalent qualifications, for the following S.R.C. C.A.S.E. awards:

1. "ESR Studies of Organo-halide Radicals" in collaboration with I.C.I. (Mond Division); and under the direction of Professor M. C. R. Symons.
2. "Kinetics and Mechanism of Pyrolysis of Silacycloalkanes" in collaboration with Dow Corning; and under the direction of Dr I. M. T. Davidson.
3. "Preparation and Thermodynamic Properties of Actinide Mixed Oxides", in collaboration with A.E.R.E. Harwell; and under the direction of Dr J. H. Holloway.
4. "Chemistry of Actinide Pentafluorides and Related Compounds" in collaboration with A.E.R.E. Harwell; and under the direction of Dr J. H. Holloway.
5. "Uses of Fragmentations of Cyclic Sulphones in Synthesis" in collaboration with Oxford Chemicals and under the direction of Dr D. J. H. Smith.

Full details and application forms may be obtained from the appropriate directors at the Department of Chemistry, University of Leicester, LE1 7RH. 2231(N)

## ASSISTANTSHIPS

### IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY DEPARTMENT OF BIOCHEMISTRY

#### POSTGRADUATE RESEARCH ASSISTANTSHIP

A Postgraduate Research Assistant is required to work with an active research group studying pancreatic endocrine function in experimental obesity. The position will be available from mid-July. Previous experience would be welcomed but is not essential.

The starting salary will be within the range £3,718 to £5,333 per annum (according to age and experience) plus £502 London Allowance and U.S.S. benefits.

Applications, including a curriculum vitae and the names of two referees, should be sent, as soon as possible, to Dr Anne Beloff-Chain, Department of Biochemistry, Imperial College, London SW7 2AZ. 2132(A)

### THE UNIVERSITY OF SHEFFIELD DEPARTMENT OF METALLURGY POSTDOCTORAL RESEARCH ASSISTANTSHIP (S.R.C.)

Applications invited from men and women scientists and engineers for a vacancy in an interdisciplinary team working on evaporation and fuming of metals. Experience of laser applications desirable but not essential. Tenable to June 30, 1981. Salary up to £4,776 a year (under review) with superannuation. Write to Dr E. R. Buckle, Department of Metallurgy, The University, Sheffield S1 3JD. Quote ref. R317/G. 2172(P)

### UNIVERSITY OF DUNDEE

#### DEPARTMENT OF MATHEMATICS

Applications are invited for a

#### POSTDOCTORAL

#### RESEARCH ASSISTANTSHIP

to work with Dr D. F. Griffiths in the above Department on finite element methods in fluid dynamics. The appointment will be available for two years from September 1, 1979 at a starting salary in the range £4,232 to £4,776 (under review) depending on age and qualifications.

Applications, which should include a curriculum vitae and the names and addresses of two referees, should be sent as soon as possible to The Secretary, The University, Dundee DD1 4HN, from whom further particulars may be obtained. Please quote reference EST/18/79J. 2196(P)

### UNIVERSITY OF BRISTOL

#### Department of Physics

Postdoctoral Research Assistantships, S.R.C. funded, are available in specific areas of polymer science relating to crystallization, structure and properties. Basic background in one of the physical sciences essential with some experience in the polymer field and/or structure techniques.

Applications to be addressed to Professor A. Keller, H. H. Wills Physics Laboratory, University of Bristol, Bristol BS8 1TL. 2216(P)

### UNIVERSITY OF OXFORD

#### Inorganic Chemistry Laboratory POSTDOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited for an SRC Research Assistant to study surface chemical reactions using Imaging Atom Probe Field Ion Microscopy. Previous experience in surface chemistry or the use of field electron or field ion emission techniques would be advantageous although of greater importance would be a desire to participate in the development and application of an interesting new technique for surface studies.

The appointment would be for two years in the first instance, preferable from October 1, 1979. The starting salary range would be £4,232 to £4,776.

Applications, accompanied by the names of two referees, should be sent to Dr G. K. L. Cranston, Inorganic Chemistry Laboratory, South Park Road, Oxford, OX1 3QR, from whom further particulars may be obtained. 2230(P)

## COURSES

### EUROPEAN SCIENCE FOUNDATION

#### EUROPEAN TRAINING PROGRAMME IN BRAIN AND BEHAVIOUR RESEARCH

#### E. T. P. WINTERSCHOOL 1980

#### "PAIN"

The E.T.P. Winterschool is a one-week lecture course which is being organised and financed entirely through the European Training Programme in Brain and Behaviour Research. The eighth Winterschool will take place in Zuz (Switzerland) from January 5 to 12, 1980. Lectures and round table discussions will be held on the central topic of "Pain".

The following speakers have been invited: S. Andersson (Göteborg); M. R. Bond (Glasgow); D. Bowsher (Liverpool); G. Carli (Siena); M. von Düring (Bochum); A. Fanchamps (Basel); H. U. Gerbershagen (Mainz); H. Handwerker (Heidelberg); J. van Hees (Leuven); A. Herz (München); A. Iggo (Edinburgh); W. Jänig (Kiel); U. Lindblom (Huddinge); P. W. Nathan (London); P. Procacci (Florence); J. Siegfried (Zürich); A. Struppler (München); L. Terenius (Uppsala); L. Vyklicky (Prague); P. Wall (London); M. Zimmermann (Heidelberg).

The Scientific Organiser of the 1980 Winterschool is Professor R. F. Schmidt (Dept. Physiology, University of Kiel).

The deadline for the submission of completed applications is September 15 and successful applicants will be notified by the end of October. Application forms and further information may be obtained from:

Dr Stephanie Zobrist  
European Science Foundation  
European Training Programme in  
Brain and Behaviour Research  
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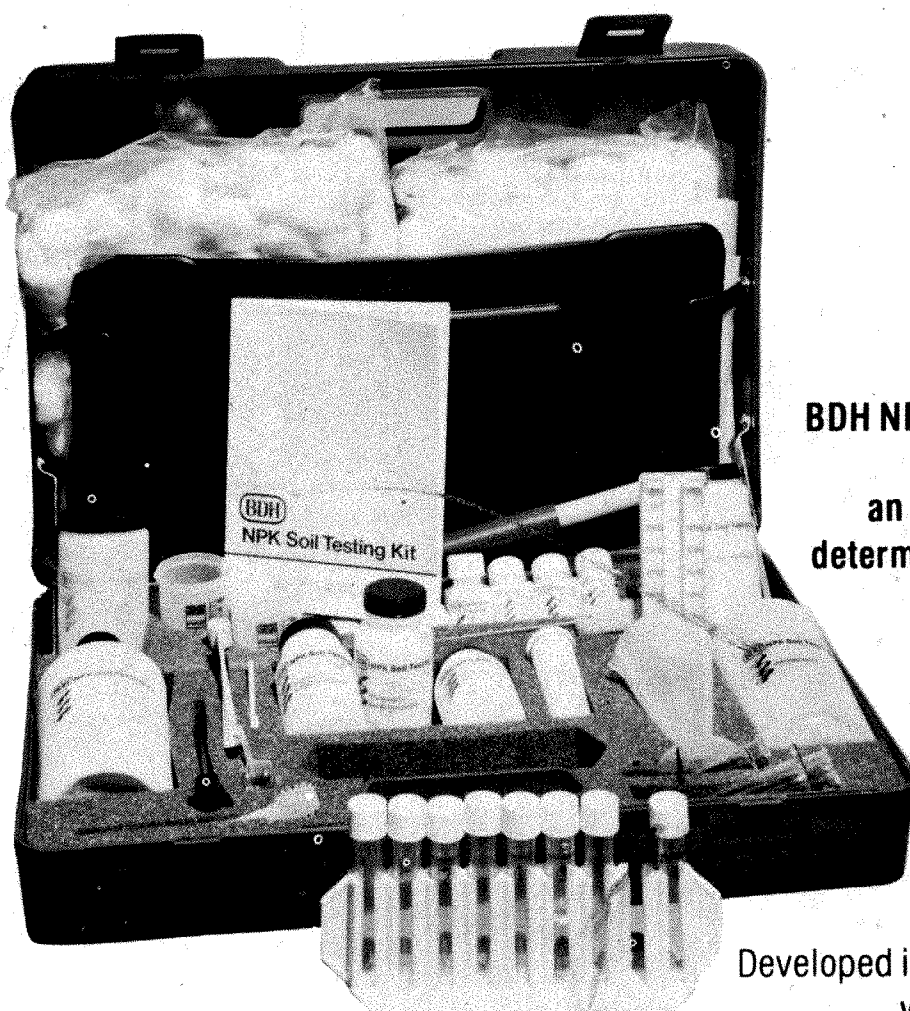
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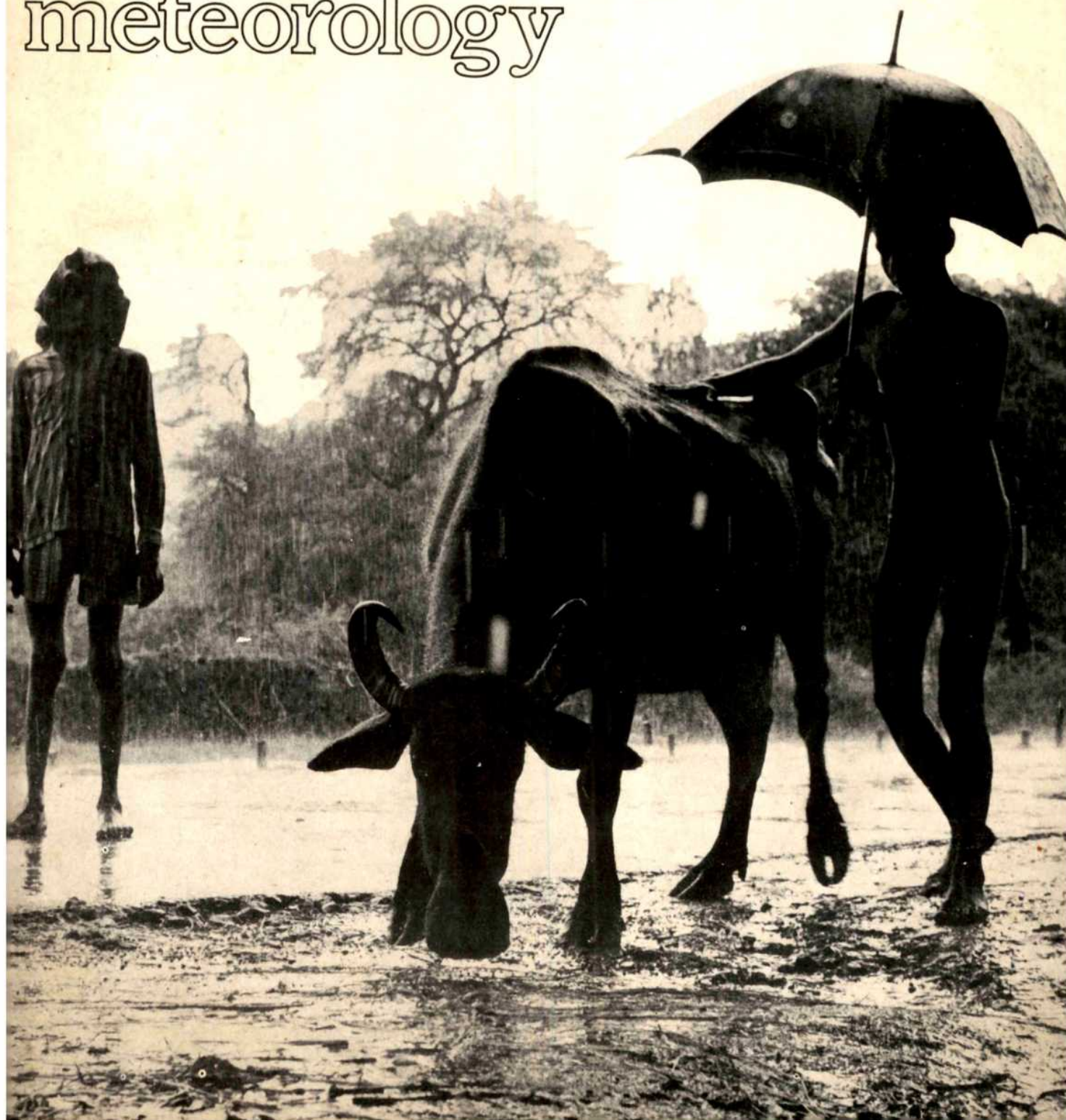
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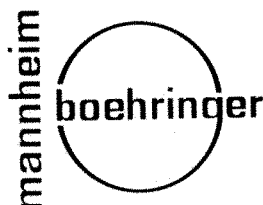
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## Cover picture

In the review article this week D. Cadet  
discusses the meteorology of the Indian  
monsoon. See page 761.

Photo: Alfred Gregory

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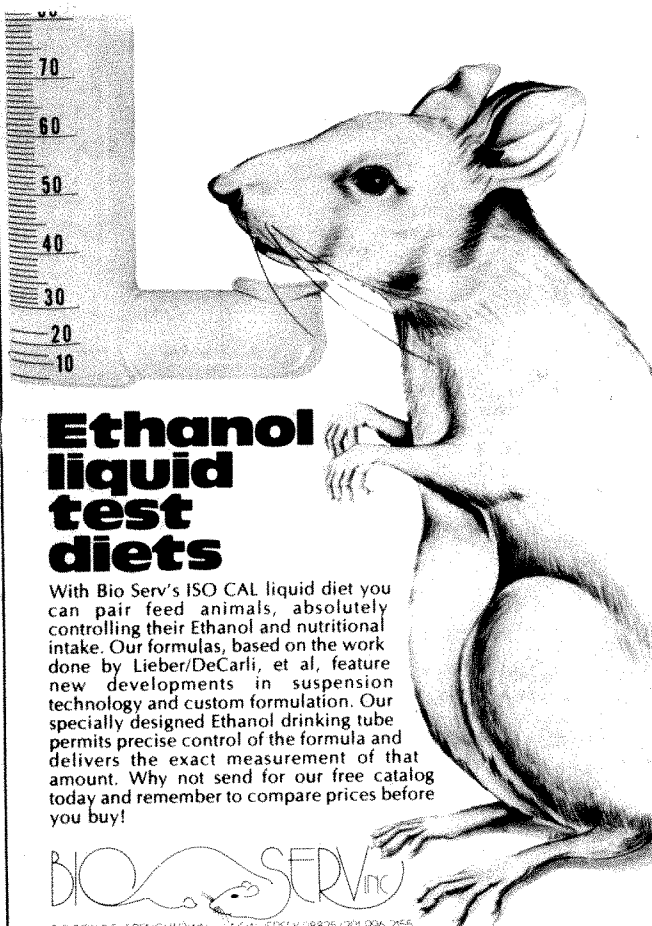
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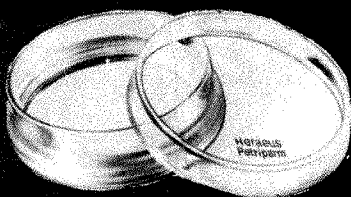
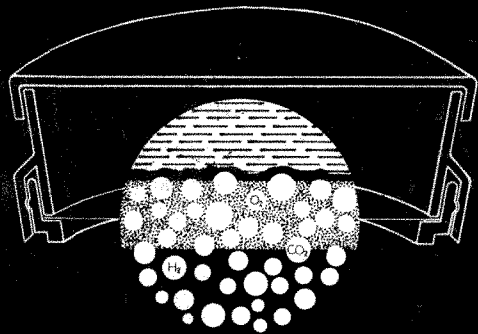
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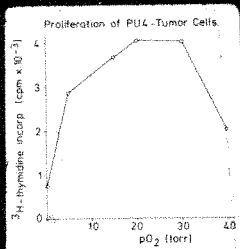
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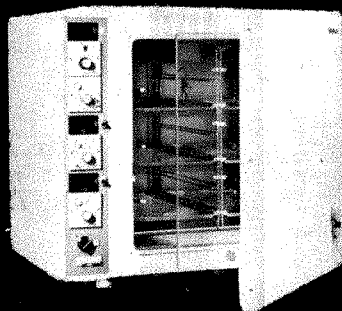
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**nature****28 June 1979**

## Problems of civil service pay comparisons

Last Friday, many tens of thousands of scientists and technologists, members of the British Civil Service, went on strike for a day. More action — withdrawal of goodwill and selective strikes — is promised by the Institution of Professional Civil Servants (IPCS), which represents about 90% of the civil servants in the relevant categories. For the majority of those who obeyed the strike call, a day away from work probably provided a welcome opportunity to catch up with some reading, secure in the knowledge that the nation's scientific and technological future is hardly put in jeopardy by a day's absence. But IPCS does have muscle; although its officials were at pains to point out that the day was one of protest not disruption, highly visible disruption there certainly was in the area of air traffic control.

Few of those involved in the strike action can have gone as far as this before — what caused the normally moderate and restrained IPCS to use its ultimate weapon? The answer, put simply, is pay, and in particular comparisons between the pay of civil servants and their counterparts outside the service. IPCS negotiates with Civil Service Department (CSD) officials (ultimately with the Minister of State for the Civil Service Department, Mr Paul Channon) over salary. There was a time when the pay for each of the many grades of scientist and technologist was established by a process of 'pay research' — painstaking comparisons were made between industry, commerce, the professions and the Civil Service in order to decide a fair level of remuneration. On the whole these comparisons were based on median salaries outside the Civil Service, but there was one significant exception — the Professional and Technological group of employees, (not the scientists), numbering about 40,000. Traditionally their pay settlements had been at well above the median of outside salaries on the basis that many outsiders, in fields such as architecture and surveying, were in business by themselves and not included in the comparisons, that there were heavy responsibilities and demands of very high quality on civil servants and so on.

Severe pay restraint from 1975 onwards meant an abandonment of pay research at a time when Civil Service salaries looked good — even better if pensions' provisions and stability of employment were allowed for. Since that time there is little doubt that the scientists and technologists in the Civil Service have lost their edge on salaries, and as pay research has gradually come back into action the extent of the increases necessary to bring civil servants back into line has become apparent. The Professional and Technology grade has been offered comparability with the median (raises of between 15% and 22%) rather than comparability with a figure well above the median — CSD rejected IPCS demands that the former grounds for favourable treatment be retained.

It had already been agreed that the Science Group (around 17,000 people) should not go back into pay research comparisons until 1980, and last year CSD declared in writing that it was 'context to accept' IPCS's proposal that for 1979 scientists' pay should be linked to that of administrators. But when administrators got an increase of 25% or more, CSD were unwilling to match the salaries for scientists. It has since done so (on 15 June) but only on condition that future pay for scientists should be based on pay research, even if this should lead to salary reductions next year, and that P & T grades would also abide by pay research (at the median, presumably).

What is behind all this? On the P & T side it is clear that the Government is unconvinced that settlements above the median are now called for, believing that they were based on a small number of special cases. On the Science side, there seem to be unambiguous warnings that the 1980 recommendations from pay research are not going to be very good news; one CSD official commented that there was a possibility that pay research, although not for implementation until 1980, 'might be relevant to the course we should take in 1979'. In other words, brace yourselves for a nasty shock.

There is a real problem here. The heart of the Scientific Civil Service is the two thousand or so Principal Scientific Officers. This is a level up to which many scientists will be moved in their late thirties but out of which relatively few will be promoted. So most PSOs will work at this grade for twenty years or more. Scientists outside the Civil Service are unlikely to be in anything like the same situation, so pay research comparisons at the PSO level (and maybe even just below it) are difficult, to say the least. As a consequence this may lead to depressed salaries.

That scientists and technologists need a pay boost is beyond doubt; the steady trickle of young computer people, for example, out of the service and into industry has to be stopped. But on the other hand salaries which look so good in comparison with industry when that industry is denuded of good people undoubtedly jeopardise Britain's industrial future. So some fine balancing has to be done.

What is in danger of being lost, however, in the heightened temperature of a strike, is the long-term need for a very serious review of the use that the nation should be making of its older scientists. It keeps them in laboratories well beyond the time many of them can (by their own admission) make an adequate contribution; their way into other parts of the Civil Service is barred, despite the benefits that numerate, scientific thinking might bring. As part of any settlement, IPCS and CSD ought to agree to look very carefully at the problem of the Principal Scientific Officer. □



# US seeks to 'rationalise' health and safety regulation in the interest of profits

The role of science in the regulatory process was a central theme in a meeting on science policy held last week by AAAS.

**David Dickson reports**

THE Carter administration is launching a concerted effort to rationalise its environmental and health regulatory mechanisms in a way that will ensure these mechanisms do not sap the vitality of the private sector. According to Mr Bowman Cutter, head of budget affairs in the Office of Management and Budget, the administration's overall aim is "to promote a structure in which decisions [about future investment] can be made rationally."

Precise details of the administration's proposals are expected to be announced shortly. Various alternatives are included among a list of possible decisions now facing President Carter on ways to stimulate industrial innovation, the results of a year-long study by the Department of Commerce.

However the general philosophy of the administration's approach was outlined by three of its principal architects — Mr Cutter, Dr Frank Press, director of the Office of Science and Technology Policy and Dr Jordan Baruch, assistant secretary of commerce — at a meeting on federal research and development policy held last week by the American Association for the Advancement of Science.

From this it emerged that a major emphasis will be placed not on ways in which direct intervention can aid innovation in private firms, but on ways of stabilising and "rationalising" the decision-making environment. And where the Commerce Department's report has provided the rationale, OMB has been studying institutional changes that will help make this possible, and OSTP looking at ways in which science and the scientific community can contribute.

Indeed having done much to set basic research funding on its feet in the first two Carter budgets — Mr Cutter told the AAAS meeting that basic science could expect a further modest amount of real growth in the 1981 budget — the role of science in the regulatory process has now become a central concern to OSTP.

Many companies blame excessive regulation as one reason for the poor performance of the US economy. "Industry has been compelled to spend more and more of its research dollars to comply with environmental, health and safety regulations — and to move away from longer-term efforts aimed at major scientific advances" is a typical complaint from Rawleigh Warne Jr., the chairman of Mobil Oil.

The problem facing the administration is that much of this regulation has been mandated by Congress. It is therefore suggesting, not less regulation as such, but that a distinction be made between necessary and "unnecessary" regulation — a distinction whose validity is questioned by many trade unionists — and that the regulatory burden be rationalised by cutting out the latter.

"Society has made decisions to repurchase clean air and water and to protect the health of workers," Dr Baruch, who was responsible for co-ordinating the Commerce Department study, told the AAAS meeting. "It is imperative that we do not sacrifice the goals of those decisions, but ask how they can be made compatible with the desire to stimulate innovation."

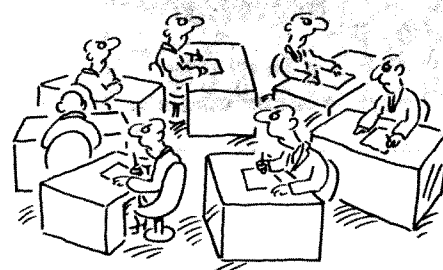
Dr Cutter described the significant impact such regulatory decisions have had on the political landscape. "Prior to the 1960s regulation was largely economic. But subsequently a very new form of regulation has begun to be developed linked to central social objectives," Mr Cutter said.

Similar points were made by Dr Press (in a speech delivered by OSTP associate director Dr Phil Smith), who criticised current regulatory efforts as being "highly-segmented, wide-ranging in impact, economically important, highly politicised, very aggressive, relatively independent, and almost totally uncoordinated."

Specific characteristics, he said, included that existence of distinct regulatory regimes, tough legislative mandates, single priority objectives with less consideration to other impacts (especially costs), the delegation of authority to separate agencies, and "a lack of any mechanism for weighing the overall impact of the sum of the separate programmes."

A major goal of the present administration, which had already implemented various activities to remedy this situation, was "improved care and rationalism, in both substance and process" he said. One area in need of improvement was the uniform application of scientific principles in the regulatory process. Another was the possible use of neutral experts to help "fence in" controversial areas so that debates on regulation "can be confined to legitimate differences in values."

University researchers could play an



"We figured the more regulators there were, the less they'd bother us — and it works!"

important role in these type of fields, he said, for example through campus based research centres. And there was no need for regulatory programmes to inhibit socially desirable innovations.

Both Dr Press and Mr Cutter admitted that rationalising the regulatory process was unlikely to be an easy task. Last year, for example, OMB had brought together seven agencies to compare their various programmes on toxic substances. But Mr Cutter admitted that the results of the exercise had been "not too successful".

This, he said, was partly due to substantial differences in objectives between the various bodies, with regulatory agencies, for example, claiming that the need to fulfil legal mandates was a principal reason for doing research, while basic research agencies gave highest priority to the expansion of knowledge.

"We will have to continue work on this. Research on the impact of regulation must increase; but before we do this, the federal government will have to get its house in order so that we know what we are spending money on and why we are doing it," Mr Cutter said.

Both speakers also agreed that the current position of "social regulation" was similar to that of economic regulation prior to the establishment of OMB by the Budget and Accounting Act of 1921, which for the first time brought the budgets of the various federal agencies together under direct presidential control.

Sceptics point out, however, that this act provided Presidents Harding and Coolidge with both the means and the authority to slash federal spending and keep it down, with total expenditures dropping from \$5.1 billion in 1921 to \$3.4 billion in 1922, and remaining low until the end of the decade. They are hoping that history will not repeat itself too closely.



## Carter aims at 20% solar energy

PRESIDENT Carter last week unveiled a package of proposed legislative measures designed, he said, towards meeting a national goal of 20% of US energy needs coming from solar and renewable resources by the end of the century.

Among the proposals are the setting up of a \$100 million solar energy bank to subsidize the interest on loans and mortgages made to home-owners and businessmen to install solar energy devices; extensive tax credits for solar installations; and the shift in emphasis from demonstration programmes of high-cost centralised solar electric technologies to "those systems which hold wider potential to displace the use of oil and natural gas".

As a result of these new programmes, the total commitment of the federal government in 1980 to solar energy will be more than \$1 billion, "a significant milestone for our country", the President said in a message to the Congress listing his proposals.

However the President's decision to fund some of the developments, including in particular the solar energy bank, from a tax he is proposing on the "windfall profits" expected by oil companies as a result of recent price increases, has been attacked by environmentalists as a political move to gather support for the proposed tax, and thus putting the solar efforts in jeopardy.

In deciding on an ambitious target of 20% solar energy by 2000 — of which one third may come from solar heating, one third from solar cells, and the rest from sources such as hydropower, windpower and the conversion of waste products into energy — the President had had presented to him a range of options, some of which placed the target much higher.

But Dennis Hayes, a research fellow at the Worldwatch Institute in Washington and a leading advocate of solar power, told a House subcommittee the previous week that the cost of the higher options, which administration officials had estimated to cost \$113 billion to reach a solar contribution of one-third of the US energy needs by 2000, had been substantially overestimated, and that in an era of fiscal frugality the high estimate had been a "kiss of death" to more ambitious plans.

The main proposals in the President's programme are:

- a 20% tax credit up to \$2,000 on new houses built to maximise the use of the sun's energy through "passive" designs
- a tax credit for commercial and multi family buildings of \$20 per million Btu saved beyond energy standards for large buildings
- increasing from 10 to 25% the tax credit for solar equipment designed to provide heat for industrial and

agricultural purposes such as drying crops

- a 15% tax credit for the purchase and installation of air-tight woodburning stoves in principal residences

- a permanent exemption from the 4-cents-a-gallon federal gasoline tax on gasohol, a mixture of 90% gasoline and 10% alcohol.

The response of environmentalist groups to the President's proposal however has been little more than lukewarm. Many of his suggestions, they point out, are already being pursued by individual legislators, while linking the solar bank to the energy trust fund, itself a highly controversial proposal in Congress, is, they claim, an unnecessarily risky move.

Many also doubt whether the amount of money which the President proposes to spend on solar energy is sufficient to head toward the goal by 2000 that he has set the nation, especially when compared to the amount of money that continues to be spent on the development of other energy resources such as nuclear energy.

"The proposed programme is a big disappointment to us; the President is talking about a minimal 5% spending on solar energy compared to other energy sources to reach an ambitious 20% goal," said Herb Epstein of the Solar Lobby, adding that his and other groups would seek to have the financial support and tax credits substantially increased as the proposals passed through Congress. □



Here comes the sun: research on solar cells using a fresnel lens at the Sandia Laboratory

## Senate vetoes plan for Third World research agency

CONSERVATIVES in the US Senate, quoting the need to restrain both public spending and the growth of the federal bureaucracy, have rejected President Carter's proposal to establish a new institute for scientific and technological research related to the needs of developing countries.

The Senate's action is not necessarily fatal to the plans for the institute, provisionally known as the Institute for Scientific and Technical Cooperation (ISTC). The House of Representatives last month defeated a similar move to kill the institute; and the legislation establishing it may therefore well be restored when Senate and House conferees meet next week to resolve differences before the relevant parts of the foreign aid bill become law.

However the Senate's decision took many of the institute's supporters by surprise, being described in terms ranging from "shock" to "disaster". "The decision is certainly very disappointing, and has effectively derailed the ISTC at the present time, although we hope that it will not turn out to be decisive," Dr Ralph Smuckler, head of the institute's planning office, told *Nature* last week.

One effect has been to cast a shadow over US preparations for the United Nations Conference on Science and Technology for Development, at which ISTC is planned to be a centrepiece in the US presentation. There is some concern that, in an attempt to appease the critics, the administration might be tempted to water down the proposal and tie it more closely to existing programmes and agencies — a move which, some fear, could seriously diminish the credibility of the institute in the eyes of many Third World countries.

It has largely been in response to inadequacies in current aid programmes that the idea of an institute specifically devoted to scientific research on Third World issues has been discussed in Washington and elsewhere since the mid-1960s, most recently in a report from the Brookings Institute.

Following these discussions, President Carter announced in a speech in Venezuela last March that he was proposing setting up a new institute with two main aims: to focus scientific effort on specific problems facing Third World countries, in areas such as health, medicine and agriculture, as well as "global" problems such as energy and the environment; and to assist developing countries in establishing their own research capabilities as a necessary step towards modernisation.

The principle of working towards these



two objectives has received wide support from virtually every sector of the US scientific and political community. The main point of dispute, however, has been over appropriate procedures, and specifically the extent to which such activities should be tied to the policy directions of other institutions.

The administration has argued publicly that although ISTC will be conceived as part of an overall development assistance strategy, the existence of a council of advisers to the ISTC's director will provide sufficient autonomy; and, privately, that anything giving greater Third World involvement in decision-making — along the lines, for example, of Canada's International Development Research Center — would fail to gain Congressional support.

During last week's debate, the Senate rejected an amendment proposed by Senator Adlai Stevenson to increase the institute's autonomy by making the directors responsible to a board of directors. Although this proposal has been widely supported in the academic community, the administration claimed that it would weaken the links to other development efforts.

Supporters of the bill had spent considerable time putting their case against the Stevenson proposal. In doing so, it turned out that they had paid insufficient attention to the threat from the other direction, namely a growing conservative constituency in the Congress pledged to cut public expenditure and the federal bureaucracy.

These were the forces that provided support for an amendment from Senator Dennis Deoncini, a Democrat from Arizona, proposing that the legislation setting up the proposed ISTC be removed entirely from the International Development Assistance Act for 1980.

"At a time when the American people are themselves struggling to make ends meet, because of spiralling inflation and the beginnings of what promises to be a substantial recession, we can ill afford another well-intentioned but expensive agency to study and coordinate the problems which are all too depressingly familiar," Senator Deoncini said.

Although the funding requests associated with the institute were not great — the administration is initially asking for an additional \$25 million to support the institute's activities in the first year, most of its budget resulting from the transfer of research projects under way in the Agency for International Development (AID) — it would subsequently devour tax dollars at an ever-increasing rate, he said. If the new agency was a response to shortcomings in AID, then improvements should be made in AID, rather than creating a "whole new bureaucracy".

Senator Deoncini was joined by Senator Robert Dole of Kansas, who referred to reports appearing in the

national press that the United Nations currently holds a substantial amount of operating funds in low-interest bank deposits. "We do not need to solve another social problem by throwing more money at it — particularly at a time when similarly destined money is presently being wasted," he said.

Supporters of the administration proposal replied to such charges by claiming that the ISTC would considerably increase the effectiveness of US aid efforts, but in the end their arguments failed to carry sufficient weight; and the amendment rejecting the institute from the aid bill was agreed by 58 votes to 42 — a margin which is said to have taken even the amendment's supporters by surprise.

"It was essentially a conservative vote, with people in the middle whose support we had previously been counting upon

arguing that the line on spending had to be drawn somewhere, and choosing this as the issue on which to do it," one administration official told *Nature* last week.

The precise fate of ISTC will not be known until representatives of the House and the Senate meet in the near future to negotiate over their differences on the foreign aid bill, in order to come up with a form that will be acceptable to both sides and can therefore be signed into law. The hope is that the conferees will agree to keep the institute in, possibly in return for cuts elsewhere.

But the restoration of funds is by no means certain. As one Washington lobbyist said last week: "Congress seems to have gone crazy this year in its desire to cut the budget; and the things that are easiest to cut are the things that are related to overseas." □



*Mutual applause: Carter and Brezhnev after the SALT treaty*

## SALT warning on MX missiles decision

ALTHOUGH urging support for the Strategic Arms Limitation Treaty recently signed by President Carter and President Brezhnev in Vienna, the Arms Control Association has warned that the benefits of the treaty will be undermined by the administration's decision to proceed with research and testing of a new generation of mobile missiles, known as MX.

In a statement released last week in Washington, the board of directors of the association say that, despite the closing of US intelligence bases in Iran, it considers the SALT II treaty to be adequately verifiable — a central issue of debate in the US Senate — and urges its ratification without substantive change.

However the ACA also expresses concern at the implications of the administration's recently announced plans to proceed with the design, development and deployment of the new MX missiles — possibly housed in and launched from underground trenches or

from holes on a "shell-game" principle — as a response to the increasing accuracy of Soviet weapons.

Criticising the lack of controls over increasing accuracy of ICBMs the ACA also says that "Deployment of more land-based, silo-destroying missiles will threaten nuclear stability and erode the basis of SALT still further if they are emplaced in mobile basing modes which multiply potential targets, forcing an adversary to programme additional warheads for targets which, in fact, contain no missiles."

The statement says that the problem of silo vulnerability is "largely hypothetical from an operational standpoint", and argues that deployment of the MX and similar systems by the USSR "will pose a far greater danger to American security, and to the SALT process and its accomplishments, than does the current prospect of a hypothetical Soviet attack on American land-based missiles." □



# Rickets under control again in UK

VITAMIN D deficiency rickets is not a disease of epidemic proportion in Britain's Asian community. Dr John Ablett, a senior medical officer in the Department of Health and Social Security (DHSS), said last week there was now enough evidence to confirm that rickets is again on the decline in the UK. The DHSS now takes the view that rickets can be eradicated completely by the use of direct vitamin D supplements to children at risk. A policy of fortifying specific target foods — such as chapati flour — is therefore not necessary.

Rickets was first documented as a serious problem in the Asian community of Glasgow 18 years ago, and since then it has been observed in most of the larger Asian communities in the UK. Despite claims to the contrary by some doctors, the DHSS says the problem can be controlled by the traditional method of vitamin D supplements backed up by a health education programme — directed at the Asian community — about rickets and its adult equivalent, osteomalacia.

The claim that rickets is on the decline is based on figures from hospital admission records. Rickets is not a notifiable disease and data collection is not easy. However, the DHSS has used figures from its Hospital Inpatient Enquiry programme — in which 10% of all hospital admissions are analysed — from over 90 areas. The declining national trend, says the DHSS, is also supported by a survey involving general practitioners in areas with a large immigrant population.

The DHSS says there is evidence that Asian immigrants are entering the country with rickets or osteomalacia, which are quite common in India, Pakistan and Uganda. This means it should now be possible to identify the population most at risk. According to Ablett, the high risk are the infant children of recent immigrants who may be vegetarians, who come from a poor rural background, and who may have a family history of rickets and osteomalacia.

The DHSS's decision that it does not want a food fortification programme is in line with the recommendation of the department's Committee on Medical Aspects of Food Policy (COMA), which has studied the problem for three years.

The composition of milk cannot be altered without renegotiations of a European Economic Community directive (see *Nature* 270, 289; 1977). Margarine has a high composition of fatty acids (46% in some brands; butter has only 10% trans fatty acids) and is causing concern to DHSS officials anyway, as these acids have been implicated in some forms of cancer.

Chapati flour, the most serious conten-



One of the healthy ones: a hospital survey showed decline in rickets in immigrant areas

der, also has problems, largely over what concentration of vitamin D to use. A concentration sufficient to prevent rickets in young children would be dangerously high for adults consuming more flour per head. This is too risky, the DHSS argues, as there is now evidence to suggest that high vitamin D intakes result in high blood calcium and may cause cardiovascular complaints and even heart attacks.

The other problem with vitamin D in chapati flour is that the vitamin is unstable, and up to 50% may be broken down in cooking processes. These technical problems with the flour have still not been resolved, and millers are reluctant to embark on a vitamin D fortification programme unless forced to do so by law.

Finally, there is the problem of hypercalcaemia. The DHSS believes — and the COMA report due to be published next year will say this — that there is now good evidence to show that high vitamin D intakes in the 1950s caused the deaths of over 200 infants reported to have hypercalcaemia. Although the DHSS acknowledges that chapati flour fortified with vitamin D would not affect this age group, it says that high vitamin D intakes could cause problems for older teenagers and young adults.

In reaching its conclusion to rely on vitamin D supplements, COMA will point out that cod liver oil supplements reduced the incidence of rickets in children, from 13% in 1943 to almost nil by the end of the Second World War in 1945. If vitamin D supplements could eradicate the problem then, the DHSS argues, that it could do the same today.

However, there may be problems on the way. Preliminary evidence from Glasgow and Birmingham suggests that some 10% of children receiving vitamin D supplements are still developing rickets. These groups will require closer scrutiny to identify why this is happening.

Alastair Hay

## UK scientists wait anxiously for £7.5m share-out

SCIENTISTS throughout Britain will learn in the next two or three weeks if they have earned a share in the Science Research Council's £7.5 million special investment in new equipment for university and polytechnic research departments. The cash distribution, which will benefit several hundred projects, follows the last government's improvements in the science budget and although the present administration has since reduced the level of increase, the SRC is committed to the move.

Originally only about £3 million had been considered for the equipment programme but the SRC has since been inundated with responses from scientists following its March appeal for applications for new research hardware. Now an expected £5.25 million alone is to go to chemistry, physics, biology and other departments covered by the SRC's science board and the remaining £2.25 million will be split between departments covered by the engineering, astronomy, and nuclear physics boards.

The decision to invest its share of the first £10 million increment in the science vote on equipment follows the SRC's prophetically accurate view that future increases were by no means guaranteed. So it was decided to spend the money as quickly as possible and grants for projects which would involve commitments to staff for several years were ruled out.

"We consider the best way that we can help universities and polytechnics to benefit from the present circumstances is to encourage them to pay particular attention to their needs for equipment", stated the March letter from the SRC to researchers.

This decision also recognised that many departments are facing severe financial problems in purchasing modern, sophisticated — and expensive — replacements for outdated instruments to provide the traditional "well-founded" university laboratory. This latter role is the function of the University Grants Committee, of course, but there are many intermediate areas between the domains of the SRC and the UGC.

Although the SRC stresses that it is not taking over any of the UGC's functioning, it is hard not to view the move as some form of help for the now financially-burdened UGC. However, in providing this new equipment, which will include items such as nuclear magnetic resonance instruments and mini-computers, the SRC is selecting those laboratories where it feels investment is best suited for its future research programme.

And the level of this need for new equipment can be judged by one estimate of £35 million for the total value of the applications received by the SRC. However



these applications were dealt with by the usual grant selection committees of the SRC and only those graded "alpha" were considered for equipment grants. Of these, about 50 per cent were selected for awards.

Now researchers are about to learn if their projects have been judged suitable.

Robin McKie

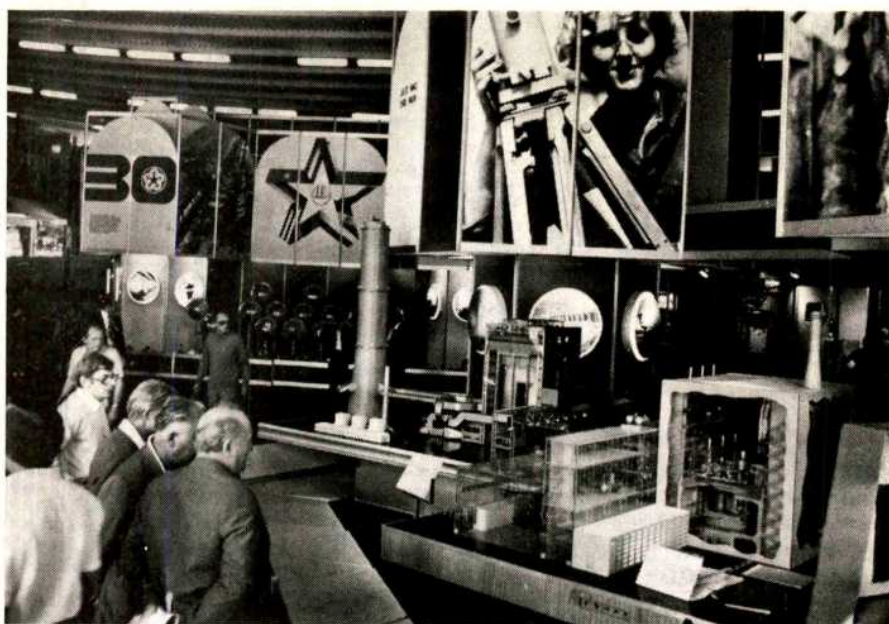
## Investors refuse to back genetic engineering

ALTHOUGH two major British firms are investing in the industrial application of genetic manipulation techniques, some academics feel the UK is not putting enough money into transferring their research work into industry. The UK has not managed to adopt the US practice of using venture capital to set up small groups of academics and industrialists who develop research to the point where it can be applied to industrial processes. "Investment in the UK is very low", according to Professor K. Murray of Edinburgh University. "Most of the use of genetic manipulation is by small US venture capital groups."

At a meeting on "new horizons in industrial microbiology" at the Royal Society last week, several industrialists responded to these criticisms by saying that venture capital was available but that "research has to pay at some time". The economic climate in the UK meant that many firms did not think it was worth taking the risk of backing a new and uncertain technology. Even if the manufacture of drugs and vaccines by genetic manipulation and, more generally, the production of alternative fuels from organic material by microbiological methods, did become technically feasible, it would be some time before the new processes were economically competitive with current technologies.

A lack of communication between academics and industry was partly to blame for the failure to transfer new ideas. Academics tended to approach industry before their research was sufficiently developed for industry to take advantage of it. Industry, on the other hand, was not willing to take up a promising piece of research where the academics had left off. The result was a gap where good research could fall by the wayside. Dr J D Coombes of Hoechst, UK thought that matters might be improved if academics did not always have to approach the National Research Development Corporation first with their ideas and if they could hold patents.

For the scientists, a major worry was that government spending cuts might affect research. "Staff levels are running down and capital expenditure is down. We will not be able to conserve the strength we already have," said Professor B S Hartley of Imperial College. □



Poznan Fair: only the Poles had their own trading agency

## Selling Polish science

THIS year, for the first time, the Polish Ministry of Science, Higher Education and Technology is appearing as an exhibitor in its own right at the Poznan International Fair, under the trading name Posteor; previously only projects developed in universities and polytechnics under its aegis have been on display.

Posteor is a financially autonomous organ of the Ministry of Science, somewhat analogous to the UK National Research Development Corporation. It was set up in 1973, a time when the Academy of Sciences first scrutinised the problems involved in the relationship between research and production. These discussions led to Poland's scheme of graduated 'problems' vital to the economy which are to be solved by science (23 November 1978 page 313); while, on the administrative side, Posteor was established to handle the implementation of new discoveries and technologies into production.

Implementation is a major problem throughout the Comecon bloc, but only Poland has adopted the idea of a special trading company. Indeed, by working through an existing foreign trading enterprise, Polservice, Posteor can earn valuable hard currency marketing know-how and licences abroad as well as protecting Polish patent rights. Through its exhibition at the Fair, Posteor hopes not only to increase its foreign outlets, but also to sell its technology and expertise at an earlier stage of development. "We can exhibit ideas, pilot technologies and know-how," explained one representative, "even if we haven't ironed out all the snags of full-scale production."

The small selection of projects on display is indeed wide-ranging: it includes a method for producing phosphates as a by-

product of sulphuric acid manufacture, and practical expertise in geodesy and water surveying, as well as an analogue system for measuring the field distribution near high voltage power lines — Poland will have its first 350 MW lines next year, as part of the Comecon supergrid. A method of cutting the petrol consumption of the average automobile by 15% underlines the importance of energy saving in the Posteor exhibits: other examples range from a cold-hardening process for worm-drive castings down to a method of freeze-drying whole potatoes. Indeed, energy conservation is a major theme of the entire Polish display, from the generating sets exhibited by Elektrim to the coal-mining pavilion with its placards extolling the leading role of the coal industry.

Following a winter when exceptional weather conditions led to a total halt in production, Poland is now rethinking one aspect of her energy policy, the proposed compensation deal with Austria. Under this agreement, Austria would build hydroelectric generating stations on the Bug, and in return would take two thirds of the current produced for the next 25 years. This scheme — like the proposal for a trans-Czechoslovakia pipeline to carry coal dust to Austria — is dear to the heart of Austrian trade minister Joesef Staribucker, and such deals are normally popular with the Polish planners because they save hard currency by paying in kind rather than cash. But it is now most unlikely to go through, for, as the Polish vice-minister of trade, Edwin Wisniewski, told journalists at the Fair: "Coal and energy are now far too precious for compensation deals. We shall always be willing to sell them, so long as we have them to sell — but it must be strictly for cash." **Vera Rich**



## news in brief

**UK government scientists to strike:** The Institution of Professional Civil Servants is continuing its industrial action through a series of selective strikes. Now scheduled for action are the government laboratories of the Agricultural Research Council and the Science Research Council. Also included will be the Central Veterinary laboratory at Weybridge, the Royal Aircraft Establishment in Farnborough and forensic scientists in the Home Office. The union is demanding pay rises linked to administrative grades for this year and an appropriate linking to outside salary structures for next year. (See leader comment.)

**US-Europe to collaborate on testing cloning risks:** The first results of a European attempt to assess some of the proposed hazards of the techniques of recombinant DNA (see p 811) help to define the way in which the next step in risk assessment should be carried out. It is a step, however, which cannot be carried out in Europe — because of the lack of animal facilities that meet the level of safety that is now required to avoid the conjectured risks that the experiment would attempt to assess. To escape from that predicament the European team will go to the US to collaborate with Dr W Rowe and his colleagues who have been carrying out a parallel study using the animal facilities at Fort Detrick. Both groups are testing whether the cloned genome of a tumour virus retains the ability to be infectious and to cause tumours. The Americans have already carried out some animal tests and will now be repeating them with the virus cloned in Europe.

**SIPRI criticises increased NATO military spending:** The Stockholm International Peace Research Institute, in its annual report, is sharply critical of NATO countries decision to increase their military spending by 3% a year. The SIPRI report states that the increased expenditure has been based on "dubious" propositions about USSR spending produced by US intelligence agencies. US intelligence has estimated that Soviet spending exceeds US spending, that it is taking an increased share of Soviet GNP and that it has been rising by 3% a year in real terms for a long time. According to SIPRI these estimates are constructed by converting labour intensive Soviet manufacturing into US capital intensive costs thus leading to an overestimate of real spending. "Valuing the military output of a much more labour intensive country such as the USSR at US prices distorts the actual situation" the report says. SIPRI also criticises the USSR policy of concealing defence spending, which it says contributes to the construction of exaggerated estimates of its spending.

The SIPRI report also warns that improvements in missile accuracy are leading to new nuclear war strategies. The old 1950s policy of mutual deterrence is giving way to a "counter-force" strategy. A new generation of missiles will be able to strike within tens of metres of hardened missile sites thus giving governments the "misplaced confidence that they can actually fight and win nuclear wars rather than simply deter them". (World Armament and Disarmament. SIPRI Yearbook 1979. Taylor and Francis Ltd, London.)

**Sussex students win reinstatement:** Sussex students Richard Flint and Shaun Fensom (21 June) were reinstated last Monday by a specially convened disciplinary committee. They had been "excluded" from campus for participating in a student union mandated examination disruption. The students were warned they would be excluded if they participated in further examination disruptions. Students have called off plans to disrupt conferences at the university this summer and the outstanding issues will be joined again in the Autumn. First year science students will decide at a mass meeting whether to continue the boycott of the preliminary science examination and the student rent strikers await the outcome of a university Senate meeting on student debt. The disciplinary committee decision followed another week of militant student action.

**US prepares for military intervention in oil crisis:** The US government announced Friday that it has established a "unilateral corps" of 110,000 men from Army, Navy, Marine and Tactical Air Force units. The corps will operate outside of NATO control and are ready for combat "in all theatres of operations where US interests are threatened". The special force will be dispersed among conventional military bases but will be mobilisable for rapid intervention at "hot points". Department of Defense head, Harold Brown said that the DOD currently is improving its sea and air transport facilities "so that we can get special units to various places distant from the US and Europe rapidly". Speaking at a press conference attended by Chief of Staff B. W. Rogers, Presidential adviser Z. Brzezinski, and Secretary of State C. Vance, Brown acknowledged that US dependence on oil imports was a serious potential "security" problem. The US gets 25% of its oil from the middle east. Brown emphasised that the US was seeking political solutions in the middle east and was consulting with moderate Arab states "which are understandably concerned about the possibility of outside intervention".

**Strict new rules on the transport of nuclear waste:** The US Nuclear Regulatory Commission recently published a strict set of rules covering the conditions under which spent nuclear fuel can be transported by road. These include the requirement that the NRC inspect and approve the route that each shipment is expected to take, that law enforcement agencies be informed in advance, and that each shipment be accompanied by guards knowledgeable about both the route, the cargo and emergency procedures.

The new rules have a dual purpose: to protect the public against possible accidents (all routes must, wherever possible, avoid major cities and urban areas), and to guard against the dangers of hijacking. To cover the latter, for example, the rules require that all vehicles be equipped with features that "permit immobilisation"; among proposals previously discussed for achieving this are dashboard buttons that would simultaneously blow out all the truck tires, explode the engine, and set off a wailing siren.

Antinuclear groups have already attacked the adequacy of the new regulations, which although they go into effect immediately, will be open for public comment for 45 days. They have challenged the first route that the NRC is now studying for approval between Norfolk, Virginia, and the Department of Energy's reprocessing and storage plant at Savannah River in South Carolina. This route would be used to transport spent fuels being brought in from abroad; anti-nuclear groups such as the Potomac Alliance argue that incoming ships could dock closer to Savannah River, and are therefore questioning the proposed route through Norfolk.

**Bellerive Group attacks pro-nuclear lobby:** The Bellerive Group, an international body of experts which includes Victor Weisskopf, a former director of CERN, has issued a stinging critique of conventional arguments in favour of nuclear power. Proponents of the orthodox pro-nuclear case have refused to answer the severe criticisms of their basic assumptions, the report says. These include assumptions about future electricity demand, patterns of energy use, and the possibility of expensive energy savings measures. Other criticisms are the inapplicability of nuclear generated electricity for transportation needs and the wastefulness of using nuclear generated electricity for space heating. The report also raises the dangers of nuclear proliferation and the possible dangers to civil liberties. The report warns that disaffection with society because of the nuclear issue "is greater than the nuclear industry and some governments that support it now realise. No free society can be governed without both trust and consent. Once trust evaporates government can be carried on only by increasing coercion".



# Urban deprivation in the heart of the Amazon

by David Bousfield

THE city of Manaus lies high on the Amazon, at the junction of the Rio Negro and the Rio Solimões. During the last century it was the centre of the rubber industry in Brazil, and the magnificent Opera House, decorated largely with materials imported from Europe, still testifies to its former wealth and importance. Since those times, however, the area has been in decline and today the State Governor complains that the development grant intended for the whole of the Amazonas region is less than that given to the Tourist Department at São Paulo in the south. Recently Manaus was made a free port in an attempt to stimulate the local economy, but despite its attraction to Brazilian tourists, who travel thousands of miles to buy cheap cameras and hi-fi, this isolated town in the heart of the Amazonian forest retains all the problems of urban underdevelopment found elsewhere in the north and north-east of Brazil.

Manaus is fortunate, however, in having on its outskirts the principal administration and research complex of the National Institute for Amazonian Studies (INPA). This department has 107 scientific staff, while the other main department, at the Museu Goeldi in Belém has 51 scientists; in addition there are a number of research stations scattered over the Amazonian area. Under the Directorship of Dr Warwick Kerr, a geneticist from São Paulo, INPA's research projects have been directed at local problems. "INPA is always canalising effort towards the benefit of the people of Amazonia", he comments. Consequently research carried out by INPA includes work on fish farming, ecologically appropriate approaches to agriculture, malnutrition, and the impact of disease on settlement along the Transamazonian highway.

The rapid development of the fragile forest environment has created many problems for urban and rural colonists alike. For example, the 3,000 km Transamazonian highway, completed in 1975, was originally intended to allow families from the drought plagued north-east to colonize the forests. Yet the agricultural advice given to these would-be farmers by David Bousfield, of the University of Sussex, held a Nature Travelling Fellowship



INCRA, the Government colonisation agency, was not based on detailed research or planning. Indeed the route taken by the highway had already been decided, and some of the first colonists settled before the main soil survey work was carried out. Upland rice was envisaged as the principal cash and subsistence crop, but INPA geographer Nigel Smith has found that a better choice would be a more diversified crop base including manioc. Diversity reduces pest damage and means that income is not dependent on the success of a single crop. Indeed Dr Smith finds that the optimal farming strategies emerging from his work bear a striking resemblance to the methods of the Indians who used to live on the land now occupied by colonist settlements.

## Mixing European and Indian crops

Since the colonists have little knowledge of which crops they should plant, additional work by INPA includes the selection of European varieties of fruit and vegetables such as carrots, cauliflowers and citrus, which are suitable for local soil conditions and climate. Attempts are also being made to reintroduce local fruit such as assaí and bacabá into the Amazonian diet, and the Indian heritage has also been useful here since many Indian tribes actively cultivated the plants which produced the best fruit. The sapota, for instance, normally produces fruit weighing 80-100g, but the Tipuna Indians have managed to increase this to hundreds of grammes, and have trees which can yield up to 11 tonnes of fruit in one harvest.

Perhaps the most interesting example of INPA's concern for the Amazonian people, however, can be found in the nearby 'favela' in the Coroado district of Manaus. This slum town is built on private land invaded by the poor some six years ago. There are now over 25,000 people living in shacks made from wood, corrugated iron and even cardboard without any form of sanitation or a proper water supply. In 1977 INPA bought one of these houses and set up a school, thus providing a valuable base for research, and for establishing some interaction between the various departments of INPA and the people of the favela. The school's name, 'Escolinha da Abelinha', which can be translated as the 'Little School of the Busy

Bee', reflects the determination and sense of humour of Dr Kerr and its first teacher and director, INPA educationalist Margie Charlwood.

By concentrating on pre-school education (children aged between 4 and 6), INPA has made a considerable contribution to the nutritional and educational development of the children of Coroado. Some 48% of the children entering State schools at 7 have to repeat their first year courses, and as a consequence many become frustrated and never go back. Pre-school education helps to avoid this early trauma, and it is a strategy now advocated by UNICEF. Even the simple measure of providing regular meals improves exam success: 73% of children in the pre-school age range suffer from malnutrition and many show signs of physical and mental retardation. By isolating the main dietary deficiencies and by trying to counteract them at the school, Charlwood and INPA nutritionist Roger Shrimpton hope to improve infant feeding habits and nutritional status.

Traditionally much of the food for Manaus has been imported. In 1975, for example, the region only produced 25% of its requirement of rice and meat, only 12% of its milk, and it imported nearly all its fruit and vegetables. The resultant expense creates some rather unexpected dietary deficiencies. Despite being surrounded by a forest containing many edible species of plant, the inhabitants of the city consume per capita only one-half the amount of fruit and one-third of the green vegetables eaten in São Paulo, and so deficiencies of vitamin A, thiamin, riboflavin and iron are common.

Bad diet preferences are also partly to blame for nutritional problems. In the days of the rubber barons the best pâté and champagne were readily available in Manaus, and today the freedom from import tariffs continues to encourage specialist tastes. The current school meals programme run in State schools and backed by the FAO includes Norwegian cod, Dutch cheese and crystallised fruit to supplement children's diets. INPA workers argue that this programme is merely creating markets for richer countries' unwanted products rather than teaching children how to feed themselves. Consequently attention is now being fo-





*Incongruous Manaus: amid the poverty, the Opera House is a reminder of former glories*

cused on the possibilities of using local varieties of fruit and vegetables.

Various diets are being introduced to the children of the Bee School and their acceptability and nutritional impact assessed. The mothers are actively associated with the running of the school and contribute to the preparation of these meals, ensuring that the educational process is not restricted to the children and the bond between the school and its community is strengthened. At the same time simple rules of basic hygiene and nutrition are being incorporated in the curriculum. An elementary reading and spelling exercise book, or 'cartilha', written by a team including Dr Kerr contains advice on which foods contain calcium and how to take care of teeth.

It is too soon to assess the project's eventual success, although the number of children attending the school has risen from 30 to 220 during its first two years, and after six weeks of the supplementary school meals their average weight increased by two kilogrammes. Once a cheap and nutritious diet has been developed, INPA would like to see the scheme extended to other 'favelas' as part of a scheme of development for the whole region.

Unfortunately the relationship between science and the solution of social problems elsewhere in Brazil is much less apparent than in Manaus. Many scientists I spoke to in the south felt that the social problems of the country (one-third of Brazil's children, some 16 million, live in slum communities) could be solved simply by political reform and government aid. But it was also clear that in the sophisticated intellectual climates of cities such as Brasilia and Rio de Janeiro a preoccupation with Western science is leaving little time for considering how the problems facing the urban and rural poor might be approached. Now Kerr and his co-workers have lead the way, will others follow? □

## Asian scientists agree on development issues

**Yonguth Yuthavong** of the Department of Biochemistry, Mahidol University, Bangkok reports on a lively meeting in Kuala Lumpur

SCIENTISTS from 21 South and South-East Asian countries met last month in Kuala Lumpur, Malaysia, and agreed on six basic principles to improve science and technology in their countries.

The meeting, organised by the Committee on Science and Technology in Developing Countries (COSTED, a body set up by the International Council of Scientific Unions), concluded that:

- Since the general level of indigenous scientific and technological capability is very low, and varies greatly among developing countries, more emphasis should be placed on regional sharing of scientific competence.

- There should be increased representation of scientists and technologists from developing countries in international bodies dealing with science, technology and development. Possible measures include travelling fellowships and the establishment of international institutions in the developing world.

- Effective and socially relevant science and technical education is one of the most important factors contributing to development. International support is needed for preparation of suitable educational material and for training of personnel, but care should be taken to promote self reliance in the education programmes of the developing countries.

- Suitable scientific and technological information systems should be established, oriented towards important issues in development.

- To make UNCSTD a success, a follow-up mechanism to implement its decisions must be speedily established.

- It was of particular importance, the meeting decided, to define the goals of development as clearly as possible. The meeting emphasised the provision of basic needs for people — rather than high per capita GNP. The Association for the Application of Science to Human Affairs (ASHA), composed of a group of scientists in India and supported by COSTED, has proposed the ASHA Development Index (ADI) based on parameters of health, literacy and employment ( $ADI = \text{per capita GNP growth rate} \times \text{employment} \times \text{literacy} \times \text{life expectancy fraction} [70 \text{ yr} = 1.00] / \text{birth rate} \times \text{infant mortality}$ ). The ADI was found to vary from 43 for poor countries to over 10,000 for rich countries. The goal advocated by ASHA is an index of over 2000 by the year 2001. While there is at present a general correlation between GNP and ADI, the meeting agreed that strategies for future development should aim to raise ADI, rather than GNP simply through rapid industrialisation with depletion of resources and urbanisation.

The lack of an effective scientific and

technological infrastructure in the developing world is reflected by various indicators: number of scientists and technologists, number of institutions, amount and quality of publications and other output. A survey of papers published in international journals showed that a very small fraction originated from developing countries. It is striking that India contributes about 5,000 papers per year (in journals covered by *Current Contents*), half of the total output from all developing countries and far more than South Africa or Brazil. The amount of research output alone therefore does not bear a simple relationship with the state of development of a country. It was generally agreed that to promote development in a poor country most effectively, not only must the research be of high quality but also the deal primarily with unique resources or pressing problems of the country. Good examples are rubber research in Malaysia and diarrhoeal diseases research in Bangladesh. This demands keen awareness of the researchers on various aspects of priority research areas — an awareness which is lacking, given the present communication gap between the scientists, the industrialists and the general population in the developing world. A remedial mechanism was proposed in which the scientists, the industrialists and the citizens could mutually interact in forums organised by the professional societies and government or private agencies. In addition, novel structures of reward and recognition should be devised to encourage research interests in development-related areas.

The meeting also emphasized the value of science as creative activity for the enquiring mind. The scientist, however, tends to become alienated from his society if his work has little relevance to its problems. A conscious effort should therefore be made by the scientific community to bring social relevance into their work, while preserving the role of making enquiry for more basic knowledge at the same time. A proper balance between basic and applied science would depend on the level of development, resources and constraints of each developing country.

It was felt that there was a need for novel scientific and technological information services orientated towards development problems of the Third World. Suitable services should be available not only for scientists and technologists, but also for administrators, field or extension workers and the general population, mostly illiterate farmers and small scale village industrialists. The problems foreseen are enormous, and



proposed solutions ranged from creation of a new information system on science and technology for development, to provision of information materials to the developing countries at a moderate cost, and promotion of public understanding of science and technology through mass media. International information agencies, scientific unions and private publishers can play substantial roles in these schemes. It was argued, for example, that sale of books and journals to the developing countries is only a minor fraction of the total sale, and the publishers should consider reducing the prices to a level which these countries can afford. Schemes for book donation such as operated by UNESCO should also be made more effective.

Nowhere is the need for public understanding of science and technology more acutely felt in the developing world than in the fields of health and agriculture. Many tropical diseases are largely preventable: hookworms are prevented by wearing shoes, cholera by having clean water supply, xerophthalmia by eating food containing vitamin A, etc. The yields of major food crops can be

increased several fold through proper fertilizer supplies, pest management and irrigation. Apart from the fact that money and supplies are simply not available, the necessary information is also not accessible to the majority of the rural population. Technology transfer for village industries also present similar problems. The development of extension services and non-formal education deserve high priority.

The strengthening of the scientific and technological capacity of the developing world will be both expensive and time-consuming. Some delegates even expressed despair that this goal will ever be significantly achieved in the majority of developing countries. An alternative goal was proposed whereby a group of countries within the same region would share their resources and pool external aid in a limited number of large and effective institutions. While this arrangement could possibly be of benefit for certain projects which need a considerable amount of scientific infrastructure, its main drawback is the continuing dependence of the smaller and less developed countries, their big brothers merely being in the same

region rather than somewhere to the North. Whatever the strategy, it was clear that much more co-operation from the developed countries should be forthcoming. This will have to include provision of scientific equipment and maintenance facilities, information, help in scientific and technical education, exchange of scientists and technologists. The developing countries themselves will have to try harder to support their scientific and technological activities, including education.

Developing countries often have an asset in abundance of natural resources, solar energy supplies and a climate suited for rapid bioconversions. The meeting agreed that these local advantages should be utilized, which implies that the problems for science and technology will often be very different from those in developed countries. It was proposed that a consortium of experts from the developing countries be formed to deal with training and development of technology for optimum utilization of natural resources. It is hoped that international agencies including COSTED will take up this matter further. □

## Hunting Mongolian dinosaurs

COMECON, in the thirty years of its existence, has provided the framework for numerous scientific cooperation projects. One of the most interesting and most unusual was the Polish-Mongolian series of palaeontological expeditions of 1963-1971, organised by Dr. Zofia Kielan-Jaworowska of the Polish Academy's Institute of Palaeozoology. (For the latest results, see p 792.)

These expeditions not only produced a wealth of scientific material on dinosaurs, dinosaur eggs and mesozoic mammals, which is gradually being published in *Palaeontologia Polonica* (the final report is expected to extend to some 16-20 thick volumes; No. 8 is now about to appear); they also resulted in an excellent "popular" book on palaeontology — Dr. Kielan-Jaworowska's *Hunting for Dinosaurs*. Most important, perhaps, as I learned when I spoke with Dr. Kielan-Jaworowska in Warsaw, they provide a fascinating commentary on how formal "scientific cooperation" can work.

Not that the Gobi expeditions were entirely typical! Cooperation agreements within Comecon tend to take place between the Soviet Union and one or more of the smaller member-countries — a major project without Soviet participation is somewhat rare. In fact, Dr. Kielan-Jaworowska explained, at the beginning there was some talk of Soviet participation, but the Soviet Palaeontological Institute was "too busy."

The idea for such an expedition arose in 1961, when the representatives of the Comecon Academies of Science met in

Warsaw. During this meeting, a cooperation was signed between the Polish and Mongolian Academies, and Dr. Kielan-Jaworowska put forward a proposal for joint palaeontological expeditions. "This was my private dream," she explained, "and also the dream of many workers in this institute. Mongolia is an unusual country, especially for vertebrate palaeontology. The odd thing is that we were invertebrate palaeontologists at the time!"

In due course, Dr. Kielan-Jaworowska was appointed to lead the expeditions. One of the main practical problems was, of course, that Poland is among the most scientifically advanced Comecon countries, while Mongolia is relatively backward. For the project to be genuinely bilateral, some preliminary training was necessary. "We had to help the Mongolian Academy," she said. "We had to teach their scientists. They came during their training to our institute and we published some joint papers. At that time there were about ten Mongolian palaeontologists working in Ulan-Bator. Two of these — Demberelyin Dashzeveg (a specialist on mammals) and Rinchen Barsbold (dinosaurs) — were very gifted."

The field-work of the expeditions proved successful beyond all hopes. Major finds included dinosaur eggs, fossil tortoises, many new genera of dinosaurs, and, for the first time, well-preserved skulls of multituberculates, which threw new light on the mesozoic "dark ages" of mammalian history. Subsequent study of the multituberculate finds showed that these non-placental mammals were closely related to



On the trail: Dr Kielan-Jaworowska (right) and colleague

the monotremes as far as brain structure was concerned, thereby raising the further question of whether they were viviparous or oviparous.

Poland has an excellent network of museums, Mongolia has simply the municipal museum in Ulan Bator. One might be tempted, therefore, to imagine that the bulk of the material will remain in Poland. "Not at all," said Dr. Kielan-Jaworowska. "The agreement is that after we have studied the material, part of it will be sent back to Ulan Bator."

"There will be a true pay-off for both sides," she added. "We have got some most interesting material, which throws great light on some of the most important questions of evolution. And the Mongolians will get good material and the basis for a good museum."

Vera Rich



# news and views

## Guest species in minerals and other crystals

from J.M. Thomas

So widespread is the occurrence of isomorphous and other forms of substitution that their nature and consequences are the concern of chemists, solid-state physicists and mineralogists alike. When a guest species is present either in small quantities or in rather large but non-stoichiometric amounts, the determination of its precise crystallographic location is seldom a simple affair. A good deal of the evidence pointing to the phenomenon of site substitution came originally, and indirectly, from a combination of simple chemical principles and accurate wet chemical analyses. It was soon established that, among the numerous kinds of silicates that constitute the rock-forming minerals, there is a tendency for  $\text{Al}^{3+}$  ions to occupy tetrahedral sites normally tenanted by Si and for  $\text{Mg}^{2+}$  to replace  $\text{Fe}^{2+}$  or  $\text{Al}^{3+}$  in octahedral sites. Similarly  $\text{Li}^+$  replaces  $\text{Mg}^{2+}$  in sites of sixfold coordination. The negative charge borne both by the framework zeolites, that nowadays play such a crucial role in the catalytic cracking and synthesis of hydrocarbons, and by the sheet silicates, which are responsible for the cation-exchange capacity of clays, is a direct consequence of this kind of substitution. Site-substitution and site-selection are also implicated in trace-metal segregation and enrichment; and thermodynamic arguments that apply to geothermometry and geobarometry (Navrotsky *Prog. solid state Chem.* **11**, 203; 1976) rely, in turn, on experimental methods of ascertaining site identity and occupancy.

X-ray crystallography, from its inception, has, in favourable circumstances, offered a more or less direct means of assessing the extent of site substitution. But in view of the factors that govern the scattering of X-rays, this approach is not well-suited to discriminate ions of elements possessing closely similar atomic numbers. Thus, whereas the pairs  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ,  $\text{Sr}^{2+}$  and  $\text{Ba}^{2+}$ , or  $\text{K}^+$  and  $\text{Na}^+$  are readily distinguishable both on grounds of scattering power and ionic radius, it often proves difficult to identify  $\text{Si}^{4+}$  sites that are occupied by guest  $\text{Al}^{3+}$ . X-ray methods encounter a similar difficulty when  $\text{Fe}^{2+}$  is replaced by  $\text{Mn}^{2+}$  or vice versa, especially if the extent of substitution is small or if the guest ions are randomly distributed within the host matrix.

In seeking alternative approaches, much progress has been achieved by spectroscopic methods, which involve monitoring the frequency, intensity and band profiles of absorptions in the infra-red and visible regions of the spectrum. Mössbauer spectroscopy, though of restricted applicability, has contributed much to knowledge of site population and preference, especially in ferrous and ferric ions in amphiboles, pyroxenes, garnets and biotites (Bancroft, Williams and Burns, *Am. Mineral.* **56**, 1617; 1971). (Conversion electron Mössbauer spectroscopy, which is even more restrictive in its applicability, has also been particularly powerful in ascertaining site characteristics in certain sub-surface phases — see Tricker, Winterbottom and Freeman *J. Chem. Soc., Dalton Trans.* 1289; 1976.) Recently, however, electron spectroscopy has burgeoned, and although its direct application has contributed little to the elucidation of site occupancy, a rather novel development (termed XPD for reasons given below) has now made possible new insights into the crystallography of various naturally occurring and chemically modified minerals. The technique is simple, straightforward and widely applicable, provided reasonably sized single-crystal specimens are available.

X-ray-induced photoelectron spectroscopy (XPS) entails the measurement of the kinetic energy of electrons liberated when a material is irradiated with monochromatic X-rays. From the known energy of the latter the binding energy of the core electron (for example, K 2s, Al 2p, Fe 3p) may be evaluated. The precise value of the core-electron binding energy is a function of the environment experienced by the atom from which the electron is emitted. But the magnitude of the 'chemical shift' in binding energy exhibited by a given ion in two or more crystallographically distinguishable sites is disappointingly small (Adams, Thomas and Bancroft *Earth Planet Sci. Lett.* **16**, 429; 1972) and inadequate to serve as a trustworthy basis for site identification. Fortunately a more subtle use of XPS can be envisaged. This entails monitoring the 'diffraction' of the X-ray-induced photoelectrons (XPD) as the take-off angle of the emitted electrons is varied. Although the existence of this diffraction has been

known for some years (Siegbahn, Gelius, Siegbahn and Olson *Phys. Lett.* **A32**, 221; 1970; Fadley and Bergstrom in *Electron Spectroscopy* (ed. Shirley) 233; North Holland, Amsterdam, 1971) its potential value in structural elucidation of the near-surface regions of solids does not seem to have been recognised previously, in spite of the early observation by Fadley and Bergstrom (1971) that the angular dependence of the XPS signal from silver dissolved in single-crystal gold was essentially identical with that of the gold. The structural elucidation of complex solids by XPD has been described recently by Adams, Evans and Thomas (*J. Am. chem. Soc.* **100**, 3260; 1978).

Suppose the angular variation of the intensity of the Si 2s photoemitted electrons of phlogopite mica is monitored. The intensity fluctuates because of the (multiple) diffraction that the electrons suffer between the point of generation inside the crystal and their escape into the vacuum. If, as was originally suggested by Mauguin (*C.r. hebdom. Seanc. Acad. Sci.* **156**, 1246; 1928) and Pauling (*Proc. natn. Acad. Sci. U.S.A.* **16**, 123; 1930),  $\text{Al}^{3+}$  ions occupy some of the tetrahedral sites normally taken up by  $\text{Si}^{4+}$ , then the angular variation of Al 2p and Si 2p photoelectrons (or of Auger electrons emitted from these atoms) should be identical, a situation which would certainly not obtain if  $\text{Al}^{3+}$  were preferentially or additionally resident in octahedral sites. By taking intensity ratios (for example, Si 2p/Al 2p or F 1s/O 1s), rather than absolute values for single core levels, sources of experimental and related uncertainty (caused, for example, by eccentricity of the specimen mount), are eliminated, and comparison of site substitution in one specimen with near- or exactly-equivalent substitution in another is rendered possible. (Moreover, the essential invariance of K 2p/2s and Si 2p/2s ratios with angle demonstrates that differences in electron wavelength and radius of ionised shell are of secondary importance.) Using this simple qualitative approach, Evans and collaborators (*Phil. Trans. R. Soc.* in the press) have re-examined critically the hitherto accepted site assignment of a range of micas. In addition to presenting direct proof that  $\text{Al}^{3+}$  ions are indeed accommodated only in the ( $\text{Si}^{4+}$ ) tetrahedral site in phlogopite, they also demonstrated that the  $\text{Mg}^{2+}$  ions

in vermiculite and the  $\text{Al}^{3+}$  ions in lepidolite are in identical crystallographic environments, both being situated at octahedral sites. Moreover, XPD shows that ions (such as  $\text{Na}^+$ ) present in muscovite in quantities too small to be detected by conventional X-ray methods, occupy sites that are similar, but not exactly equivalent, to those occupied by the interlamellar  $\text{K}^+$  ions which they replace.

By its nature the technique is more reliable the smaller the extent of substitution, and it does not require the guest species to be ordered (contrast X-ray diffraction). And although it is biased in favour of the sub-surface regions (about 100 Å) of the crystal — because of the magnitude of the electron escape depth — it possesses further advantages in that, combined with measured XPS intensities and known photoelectric cross-sections, it affords insights into the degree of hydration of exchangeable cations. Thus it has been established (Evans, Adams and Thomas *Phil. Trans. R. Soc.* in the press) that, whereas interlamellar  $\text{Ca}^{2+}$  or  $\text{Pb}^{2+}$  ions in weathered or solution treated vermiculite are shrouded with a hydration shell, interlamellar  $\text{K}^+$  ions, on the other hand, are bare. In similar vein, Evans (in preparation) has recently shown that substitutional titanium ions in the mineral biotite occupy both tetrahedral and octahedral sites (the approximate percentage population being 30 and 70 respectively). Furthermore, he reported at the European Conference on Surface Science (ECOSS2) in Cambridge, March 1979, that, by recording intensity variation as a function of azimuthal angle (at a fixed polar angle) information about site symmetry (of the photoemitting atom) may be gleaned. In this respect, XPD is formally akin to the more strictly surface tools that utilise angular variation of core-level electrons liberated (by X-rays or synchrotron radiation) from adsorbed phases. These tools have been applied with conspicuous success by Kono, Fadley, Hall and Hussain *Phys. Rev. Lett.* **41**, 117; 1978) who succeeded in extracting information on both the symmetry and bond lengths associated with oxygen chemisorption on copper, and by Woodruff, Norman, Holland, Smith, Farrall and Traum (*Phys. Rev. Lett.* **41**, 1130; 1978) who arrived at quantitative surface structural information relating to the adsorption of Te and Na on Ni(100) faces.

It seems clear that, provided experimental methods can be evolved to cope with smaller single-crystal specimens, a wide range of structural and thermodynamic problems in geochemistry, as well as in materials and engineering science, should be clarified by the application of XPD. □

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## Proline and folding proteins

from Robert Freedman

A FOUR-YEAR-OLD hypothesis that the imino acid proline has a special role in the folding up of proteins has been confirmed, refined and extended by several, almost simultaneous publications from laboratories in Britain, the United States, Switzerland and the Federal Republic of Germany. One of the papers gives a detailed picture of how the complete three-chain protein collagen, the main protein in tendons and cartilage, folds itself up. Proline is unusual among the components of proteins in that it is an imino acid rather than an amino acid. This gives it unique and long-recognised properties of restricting the folded structures a protein can take. But it has become apparent recently that prolines may also control the rate at which proteins can fold up from an unfolded state.

Thirty years ago, Linus Pauling showed that in most peptide bonds, the side chain groups of the amino acids ( $\text{R}_1$  and  $\text{R}_2$  in Fig. 1) are held as far apart as possible in a *trans* configuration for the peptide bond. This is much preferable to the more crowded *cis* arrangement. But for bonds involving the NH group of proline both *trans* and *cis* arrangements are possible; the *trans* configuration is still slightly preferable and is found more often in folded proteins, but in an unfolded protein the *cis* form of the bond could easily occur. If that were the case then the process of getting proline peptide bonds into the right configuration would be important in the folding up of proteins.

In 1975, Brandts, Halvorson and Brennan (*Biochemistry* **14**, 4953) collected data on the folding of proteins and pointed out two important facts. For many proteins the folding process occurs in two phases and the rate of the slow phase is very similar to the rate at which proline peptide bonds isomerise — interconvert between *cis* and *trans*. To explain this, they proposed that only the unfolded molecules with all their proline-containing peptide bonds in the correct configuration could fold up. So the fast phase corresponded to the folding of the molecules which were all correct at the start of folding, while the slow phase corresponded to the slow isomerisation of the remaining molecules.

Gradually the data have accumulated to support this interpretation. Over the years Baldwin and colleagues at Stanford have analysed the slow and fast phases of refolding of ribonuclease in great detail. Now, he and Schmid have shown that the slow phase is the result of slow isomerisation of peptide bonds involving proline, but their quantitative data do not exactly agree with the model originally put forward (*Proc. natn. Acad. Sci. U.S.A.* **75**, 4764; 1978). This proposed that all the peptide bonds in the unfolded protein would have to isomerise to the correct configuration before folding could begin. The data of the Stanford group suggest that not all the bonds need to be correct; there may be some 'permissive' proline residues which can be fitted in, either in the *cis* or the *trans* configuration.

Similar modifications of the theory have been suggested by Creighton, who has considered the problems facing a large protein containing a lot of proline residues (*J. molec. Biol.* **125**, 401; 1978). Whereas the slow phase of refolding for the small proteins mentioned above is of the order of a few seconds, Creighton shows that for a protein containing 20 proline residues it would be about 10 min, and would stretch into hours for a protein with 30 proline residues. These figures are so unreasonably large that modifications to the model have to be considered. There are two related possibilities. The protein could be divided into domains each of which can fold independently; the folding of each domain would be as fast as that for a small protein and the whole molecule would soon be fully folded. Alternatively, without a clear division into domains, a protein could nevertheless fold itself up bit by bit, the region around a 'correct' proline folding to its final conformation without having to wait for all the prolines in the molecule to be in the right configuration.

The former of these proposals, domain organisation, is probably what happens in most globular proteins. But for collagen, a fibrous structural protein, there is now direct evidence for the second — 'bit by bit' — hypothesis, from the work of Bächinger, Bruckner, Timpl and Engel (*Europ. J. Biochem.* **90**, 595, 605; 1978). This collaborative group based at Basle and Martinsried has isolated a fragment of procollagen which on a small scale resembles the whole collagen molecule. This fragment (peptide Col 1→3 of bovine type III procollagen) consists of three poly-

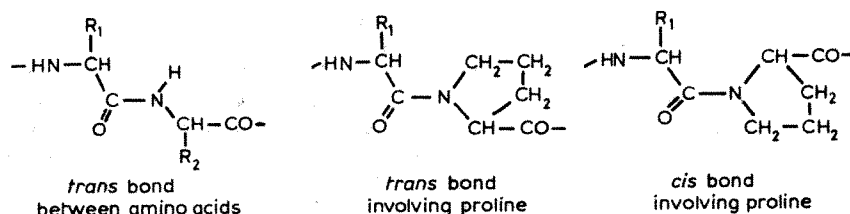


Fig. 1

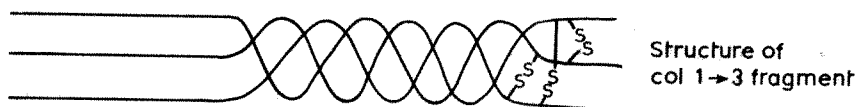


Fig. 2

peptide chains, the central regions of which are twisted round each other in the 'triple-helical' structure characteristic of collagen. At the end of the triple-helical region, the chains are fixed to each other by a kind of knot formed by disulphide bonds between the chains (Fig. 2). Bächinger *et al.* have unfolded this fragment and studied its folding as a model of the folding of collagen itself.

The interesting point here is that the Col 1→3 fragment, like collagen itself, is very rich in proline residues and hydroxyproline residues. The whole fragment has 27 such residues in each of its three chains so that they make up 20 % of the protein; in the triple-helical region they comprise one-third of all the residues. But Col 1→3 folds into its complex triple-helical structure in not much more than a minute. Is this speed consistent with the hypothesis that proline peptide configuration is crucial to folding?

The group from Martinsried and Basle showed that peptide bond isomerisation was important by a neat experiment. When they refolded Col 1→3 from the unfolded state it folded in two phases. But the proportion which refolded fast was greatest when they used material which had only just been unfolded. In other words, if you unfold the protein and immediately try to refold it, most of it can refold rapidly;

but if you wait before refolding it, the proportion which refolds rapidly, diminishes progressively. This suggests that immediately after unfolding all the bonds are in the correct configuration for folding, but that they gradually isomerise and incorrect configurations accumulate. All the thermodynamic parameters of the folding and unfolding also showed that the isomerisation of proline peptide bonds was the rate-determining step.

So how are all the proline peptide bonds isomerised so rapidly? The answer is that the process can occur bit by bit. The disulphide knot is a nucleus from which folding can begin. Then the three chains can begin to twist themselves up correctly until they come to a proline in the wrong configuration. Once that has isomerised they can proceed as far as the next wrong one and hence the folded structure is rapidly propagated. Because the protein here is essentially a linear cable, rather than a tangled mass like globular protein, folding can proceed in this stepwise manner, rather than being an all-or-none process which depends on all the proline residues being in the correct configuration at the start. □

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## West African membrane controversy

from Peter J. Smith

SOME years ago Turcotte and Oxburgh (*Nature* 244, 337; 1973) introduced the fascinating concept of 'membrane tectonics' to explain certain aspects of the internal tectonic behaviour of the Earth's lithospheric plates. The idea is simply that plates, being thin shells in relation to the Earth's radius, should act as membranes, the theory of which has long been familiar to civil engineers and the like. Specifically, as the Earth is an oblate spheroid rather than a perfect sphere, any plate moving from one latitude to another will experience a change in curvature and all that results from it. Generally a plate travelling away from the equator will find its curvature decreasing and its periphery under tension, whereas a plate approaching the equator will increase its curvature and experience peripheral compression. A moving plate will thus be subjected to deformation, stresses and consequent secondary phenomena such as fracturing.

As possible examples of membrane tectonics in action Turcotte and Oxburgh suggested Hawaii and East Africa; but later Freeth (*Earth Planet. Sci. Lett.* 38, 298; 1978) was to put forward what he held to be "an even better example", namely, West Africa and the Gulf of Guinea. It is difficult of course to apply simple membrane theory to a plate containing an inhomogeneous continent with an irregular outline, not least one that for more than 100 M yr has been growing (by accretion at the mid-Atlantic ridge) and parting from a once-joined continent (South America). Nevertheless, Freeth managed to convince himself that in the West African rift zone (chiefly the Benue, Yola and Gongola rifts), compression and tension occurred in the way one would expect for a region first approaching the equator and then receding from it in the northerly direction.

Thus as the West African rift system

moved towards the equator from the south 90–40 M yr ago it underwent compression, resulting in folding, the eruption of andesites (implying subduction) and the development of mylonite zones. But while the region was in the vicinity of the equator about 40 M yr ago there was little or no tectonic activity. Then during the past 30 M yr or so, as the area has moved away from the equator, there have been new rifting and volcanic activity, most notably those associated with the development of the so-called Cameroun volcanic line extending from the offshore islands in the Gulf of Guinea into central Cameroun.

Freeth's presentation appeared fairly convincing in a hand-waving sort of way, or at least it was something worth thinking about. But it failed to convince Thorpe and Wright (*Earth Planet. Sci. Lett.* 42, 327; 1979) who have now objected to it on two main grounds. They point out first that during part of the crucial period up to 40 M yr ago, when the Benue rift was supposedly under membrane compression, the region was actually under net tension — an unexplained inconsistency acknowledged by evidence quoted by Freeth himself. Moreover, during the past 40 M yr, while the region was supposedly under membrane tension as a result of drift away from the equator, it may not have been moving northwards at all. The palaeomagnetic evidence appears to be more consistent with an Africa more or less stationary during this period, an interpretation that opens the way to the alternative hypothesis that "volcanism and tectonic activity are consequences of the focussing of sub-lithospheric thermal anomalies onto a slowly-moving or stationary plate".

The second point made by Thorpe and Wright is that membrane theory postulates peripheral or marginal compressions and tensions in moving plates, a geographical factor that Freeth first acknowledged but subsequently appeared to ignore. The Gulf of Guinea may have been plate-marginal when Africa and South America first separated, but ever since then it has in effect been migrating further into the African plate as the South Atlantic has grown. Nor is the volcanic activity associated with the mid-plate setting of the Cameroun volcanic line unusual in the context of the African continent as a whole; such activity occurs throughout the non-cratonic areas of North Africa and is not even preferentially distributed towards plate-peripheral zones.

A third, rather less central, point made by Thorpe and Wright is that "there is no evidence for any sea-floor spreading and subduction in the Benue trough". This, as students of the region will know, is a highly contentious point. In his response Freeth (*Earth Planet. Sci. Lett.* 42, 329; 1979)

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devotes considerable attention to it; and if the power of detailed referencing and a sense of conviction were the sole criteria, he would win hands down. It is a pity, though, that in following up this issue Freeth fails to do justice to the two more crucial criticisms. He makes no mention at all of the question of the marginality of compression/tension. Nor does he pursue the problem of compression or tension prior to 40 M yr ago. On the other hand, he does deal with the continental drift issue, pointing out quite rightly that the palaeo-continental maps he used, namely those of Smith (A.G.) and Briden *Mesozoic and Cenozoic Palaeocontinental Maps*, Cambridge University Press, 1977) incorporate the most comprehensive data set available.

So where does that leave West African membrane tectonics? In a highly unsatisfactory state, unfortunately. As far as the impartial observer is concerned, a reading of the current literature on the controversy suggests that the opposing cases are equally good, or rather equally bad. In the credibility stakes Thorpe and Wright have the edge; but that may be because by the very nature of things it is easier to put a spanner in the works than build a nice bit of machinery. The moral is that it would probably be better to look to a less complex area if the aim is to assess the reality or otherwise of membrane tectonics. If the object is to explain the evolution of the West African rift system, on the other hand, someone is going to have to put forward some rather more convincing evidence one way or the other. □

## A diet of worms

from J.E. Sulston and J. Hodgkin

FIVE years ago Brenner published an extensive genetic characterisation of the small free-living nematode *Caenorhabditis elegans*. Largely as a result of his pioneering work, this organism has become the subject of many different lines of research. Last May more than 120 researchers met at Cold Spring Harbor to discuss recent findings in *C. elegans* biology\*.

This animal is a self-fertilising hermaphrodite whose rapid life cycle (3.5 days) facilitates genetic manipulation. Its small size (1,000 µm × 60 µm at maturity) and transparency facilitate the observation of cells *in vivo* and also make possible the use of whole mounts for histology. The subsequent location of defined regions and serial sectioning for electron microscopy are now routine. Development is predominantly mosaic, and because of the small and invariant number of cells (550 at

hatching), the behaviour of cells in the wild type can be defined exactly. The genome is small (haploid DNA content twenty times that of *E. coli*) and estimates of the number of essential genes are low (2,000-4,000).

Perhaps the most important advances reported at the meeting were in the isolation of cloned DNA fragments, in the refinement of genetic methods, and in the characterisation of developmental mutants. With these tools, progress towards analysis of the genes controlling development seems feasible.

S. Emmons (University of Colorado, Boulder) described work on randomly cloned restriction fragments: detectable differences between the Bristol and Bergerac races of *C. elegans* could be demonstrated for 15 % of these fragments, and such fragments can therefore be assigned to particular regions of the genetic map. No differences between germ line and somatic DNA were detected for 50 different fragments although such differences (chromatin diminution) are known to occur in certain other nematodes. Analyses of ribosomal DNA (J. Files, Boulder) and of three tRNA genes (T. Tranquilla, MRC Laboratory of Molecular Biology, Cambridge, UK) were reported; the repeat length of the ribosomal DNA is short (6,800 base pairs).

Muscle assembly can be studied both biochemically and genetically in *C. elegans*. A MacLeod (Cambridge, UK) has cloned a cDNA fragment corresponding to the major myosin heavy chain species. The structural gene for this protein, *unc-54*, has been subjected to detailed genetic analysis by R. Waterston (Washington University School of Medicine, St Louis), who presented a fine structure map, and by P. Anderson (Cambridge, UK), who has obtained more than 100 new *unc-54* mutations by a selective technique. Some of these mutations are small deficiencies that allow mapping of the genes adjacent to *unc-54*.

At least 17 other genes affecting the body wall muscle structure are now known, which map throughout the genome (J. Zengel, Baylor College of Medicine, Houston; Waterston, St Louis). Fine structure maps of two of these genes, *unc-15* and *unc-22* were presented by A. Rose and D. Moerman (Simon Fraser University, Burnaby): *unc-15* is the structural gene for paramyosin, but the product of *unc-22* is unknown. It may interact with the *unc-54* (myosin) product since some *unc-54* mutants suppress some *unc-22* mutants. This suppression appears to be specific. On the other hand, two pleiotropic suppressors, *sup-5* and *sup-7*, have been identified that suppress mutations in a wide variety of genes (Waterston and others). Only null alleles appear to be suppressed, and the mechanism of suppression may well be translational.

E. Schierenberg (Max Planck Institute for Experimental Medicine, Göttingen)

reported progress in defining the complete embryonic cell lineage; this has now been described up to the 220 cell stage, and some lineages have been followed considerably further. The Göttingen group has also begun to characterise temperature sensitive lethal mutants that alter embryogenesis at the non-permissive temperature; some of these have abnormalities in the timing of cell divisions and in the movements of embryonic cells. The stages at which development is arrested have been described for these mutants, and also for a set of non-conditional lethal mutations isolated by P. Meneely (University of Minnesota, St Paul). Mutations altering post-embryonic cell lineages have been found: a number which affect vulval development were described by C. Ferguson (Massachusetts Institute of Technology). In two other mutants certain cells fail to undergo terminal differentiation and continue to divide in a stem cell mode instead (M. Chalfie, Cambridge, UK).

There is increasing interest in the experimental manipulation of development. Isolated blastomeres can now be cultured to the point where intestinal and muscle cells have differentiated (P. Bazzicalupo and J. Laufer, Boulder). The fate of the blastomeres is largely independent of the presence of their neighbours, and after blockade with cytochalasin and colchicine, characteristic intestinal granules develop only in those blastomeres that would normally give rise to the intestine. By both these criteria early development is mosaic. On the other hand, definite episodes of regulative development after hatching have been demonstrated by cell ablations with a laser microbeam (J. Kimble, Cambridge, UK) although here too development is predominantly mosaic. In further analysis of these events cell specific markers will be of great importance. Suitable probes are cloned cDNA preparations which can be used for *in situ* hybridisation to mRNA (K. Edwards, Boulder), antibodies to specific cell products (M. Klass, Boulder), monoclonal antibodies (Y. Argon, Carnegie Institution, Baltimore) and plant lectins (E. Hedgecock, Cambridge, UK).

Many other investigations were reported at the meeting but cannot be described here in detail, including work on ageing, cuticle structure, dauer larva formation, egg laying, fertilisation, hormones, intermediary metabolism, neuropharmacology, nutrition, sex determination, sperm motility, and tail formation. There were relatively few presentations on the neurobiology of *C. elegans*, although almost all of the wild type neuroanatomy has now been reconstructed. Work on the related but much larger nematode *Ascaris* was reported by J. Donmoyer and J. Walrond (University of Wisconsin, Madison); the organisation of the ventral

\*The Second International *Caenorhabditis elegans* Meeting, supported by the US National Institute of Aging and National Science Foundation was held on 10-13 May 1979.

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cord neuroanatomy appears to be similar in the two species, and electrophysiological experiments (impossible in *C. elegans*) have led to the formulation of a model for bend formation in *Ascaris*. The same two classes of acetylcholinesterase are found in both nematodes, and both are widely distributed in *Ascaris* (C. Johnson, University of Wisconsin, Madison). The elimination by mutation of the two classes (but not either class alone) leads to defective movement in *C. elegans* (J. Culotti, Göttingen).

The participants seemed to be united in their enthusiasm for *C. elegans*, and their interest in all aspects of its biology. Specialisation has not led to fragmentation. □

## Nonsolar planets and their detection

from David W. Hughes

I AM never sure which is the more surprising supposition — that our planetary system is of such extreme rarity that it might be unique in the Galaxy — or that planetary systems similar to ours exist in orbit around one in every four stars. There seems to be no inherent reason why Earth should not be the only home for living creatures, an assumption that obviously makes our search for extraterrestrial life futile. On the other hand planets may well be common. There seems to be no sharp distinction between binary and multiple star systems and stars with planetary companions. The masses of small stars in binary systems seemingly grade continuously down to the masses of planets. The obvious solution to this dilemma is 'look and see'. The discovery of nonsolar planets would provide a vital clue to the origin of the solar system, a subject which is bedevilled at the present by the fact that only one solar system can be studied in detail. Bracewell and MacPhie have reviewed the problems inherent in searching for nonsolar planets in a recent edition of *Icarus* (38, 136; 1979).

Astrometry is one of the existing techniques which, according to the authors, "with sufficient refinement may detect nonsolar planets". Plate scales of 15 arc sec per mm can be obtained with very long focus refracting telescopes and these enable very small angular displacements of stellar images to be detected. A year's observation gives a precision of 0.003 arc sec. The problem is exemplified by the fact that an observer of the Sun from a distance of 10 parsec would be looking for an epicycloid wiggle of amplitude about 0.0005 arc sec and period of about 12 yr just to detect Jupiter. Needless to say, many researchers regard the astrometric technique as already sufficiently refined to detect planets. Van de Kamp and

Lippincott of the Sproul Observatory have reported the discovery of invisible planets around Barnard's star, Epsilon Eridani and Ci 2354. Lalande 21 185 is also thought to have a planetary companion. It is interesting to note however that unlike the near circular orbits of our planets these objects seem to have high eccentricities, a factor they have in common with double stars.

Orbiting planets can also produce a sinusoidal variation in the stellar radial velocity. Observed in the ecliptic plane and from a distance, the amplitude of the variation for the Sun is  $12 \text{ ms}^{-1}$ , equivalent to a  $3 \times 10^{-5} \text{ nm}$  shift in the 656.28 nm Fraunhofer line of the solar spectrum. Present practice achieves a precision of several hundred metres per second so this is a technique for the future.

When the combined light of Jupiter and the Sun is viewed from say 10 parsec only about one in  $6 \times 10^8$  photons received actually comes from Jupiter. Also light from the Sun, diffracted by the edge of the telescope aperture obscures the planetary light. Diffraction may be overcome by shading the aperture, a process known as apodisation in which light transmission falls off gradually from the centre of the field towards the edge. Such a telescope, in orbit above the atmosphere can theoretically detect planets.

Infrared long baseline interferometry can be used. Planetary thermal infrared radiation might not be strong but at least it comes from near the peak of the spectral distribution. For stars, the infrared spectral region is well down from the peak of the spectrum. The ratio between stellar power and planetary power drops to around 5,000 at wavelengths longer than about  $20 \mu\text{m}$ . As the distance between the elements of an interferometer is increased, fewer and fewer objects can be detected. The ones that can are those with the smallest angular diameter and this of course favours planets. Bracewell and MacPhie consider possible infrared observations that can be obtained by future space probes. The first one entails having two orbiting infrared ( $40 \mu\text{m}$  say) collectors separated by 44 km (that is  $10^9$  wavelengths). The angular diameter of the star is then about five times the interference fringe spacing so as the star changes its position relative to the fringe pattern the receiver power hardly varies. The planet however is about one tenth the stellar angular diameter and fringes sweeping over the planet produce a substantial modulation. Unfortunately the observing advantage gained is only about 20, which does little to reduce the previous factor of 5,000.

An improvement can be made by placing a minimum of the interference pattern on the star. The planet is then placed at the

adjacent maximum, this requiring, at  $40 \mu\text{m}$ , a base line of 7.7 m for an angular separation of  $2.6 \times 10^{-6} \text{ rad}$  between star and planet. The star to planet power ratio drops to an amazing 1/80. This signal can be modulated, and thus more easily detected against the background, by spinning the interferometer about an axis through the star. Unfortunately the basic pointing accuracy required is 0.001 sec arc. For a detector of area  $1 \text{ m}^2$  and a receiver of bandwidth  $\Delta\lambda$  equal to  $0.1\lambda$  a planet like Jupiter 10 parsec away would produce a flux of 2 photons per second. A reasonable signal to noise ratio is obtained with 10 h of observing time. Unfortunately again, the particles that scatter the zodiacal light produce a strong infrared background and the proposed detector would register 150 photon  $\text{sec}^{-1}$  from this source. To measure the planetary signal against this large background to an accuracy of a few per cent would take a month.

The device proposed by Bracewell and MacPhie has two off-axis paraboloids producing the interference null. Tracking control uses the interferometer optics but at visual wavelengths, the infrared and visual beams being separated by 45 degree plates. The critical components of the detector will be kept at liquid or superfluid helium temperatures, less critical parts having to make do with the 30 K temperature of the triple point of neon.

Obviously this instrument is utilising all the hoped-for technical improvements. It is also apparent that all our attempts to detect non-solar planetary systems rely so far on the observation of minor fluctuations in large quantities. We will have to wait a long time before we get a high definition view of another solar system, an agonising wait which does little to stifle our feeling of loneliness in the universe. □

## Reconstructing the past

from Peter D. Moore

IT is the task of the palaeoecologist to make mental reconstructions of past environments on the basis of such evidence as assemblages of fossils. First the assemblage must be interpreted in terms of contemporaneous communities of organisms; second, the environment is reconstructed from what is known of the ecology of the organisms involved. The Quaternary palaeoecologist would seem to have certain advantages at both these interpretive stages as most of the species with which he deals are still extant and can therefore be subjected to various types of community analysis and other types of ecological enquiry. Just how real these advantages are may be questioned, for

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many problems of interpretation remain at both levels of analysis.

These problems are well illustrated by the current concentration of attention on the closing phases of the last glaciation. Of particular interest is the climatic event which took place between about 11,000 and 10,000 yr ago, which involved the reversal of the trend towards increasing warmth and deglaciation, and resulted in the re-advance of glaciers in Scandinavia and Scotland and the reformation of local corrie glaciers in outlying mountainous districts. This event (often referred to as the 'Younger Dryas' after the discovery of remains of the plant species *Dryas octopetala* within sediments where the episode was first described), was very localised on a global scale.

Some views of the conditions prevailing during these times were expressed at a meeting of the Quaternary Research Association at University College, London in January. W. A. Watts (Trinity College, Dublin) showed that the biological changes induced by the event were pronounced and of both qualitative and quantitative character in the maritime fringes of North-West Europe, as in the western British Isles, but were weaker and became increasingly difficult to detect in fossil assemblages as one moved eastwards and southwards across Europe. In the area of the Alps there are only small, quantitative changes in flora during the Younger Dryas together with some sediment changes indicating increased erosion. South of the Alps, in the Po Valley, there are no sediment changes and the entire event is difficult to detect on the basis of the pollen record.

Such geographical variations in the fossil record of the time illustrate the local nature of some apparently profound climatic changes. In the case of the Younger Dryas event, it suggests that the climatic cause was associated with oceanic air or water masses, and Watts drew attention to the marine sediment evidence of a more southerly location of the Polar water mass front in Younger Dryas times.

Having established the geographical and temporal pattern of this climatic deterioration, one is then faced with the question of the precise sequence and nature of climatic conditions within its span.

Evidence from benthonic ostracods was presented by J.E. Robinson (University College, London) for the shelf waters of North-West Europe. Here the Younger Dryas event is marked by a decrease in species richness, which is now a characteristic of Arctic waters, and also by the occurrence further south of ostracod species now restricted to the Arctic Ocean, such as *Rabulimys mirabilis* and *Krithe glacialis*. This use of indicator species is an interpretive technique commonly used by palaeoecologists, which is based on the assumption that the factors which determine current distribution patterns of extant organisms are the same as those

which limited their distribution in the past. In this case temperature is thought to be the critical limiting factor. Although attractive as a rule of thumb, there are several problems associated with the use of this line of reasoning. Most physical environmental factors are complex in their diurnal and seasonal variations and it is only rarely that one can pinpoint the interaction between such a factor and the life history of an organism which results in the limitation of the organism's range. Also, such relationships are often made complex by the involvement of other species, such as prey, predators, parasites and competitors. Because such communal relationships can vary in time, the physical factors currently coinciding with an organism's range may not have so coincided in the past.

This is certainly not a problem confined to ostracods. Work on beetles presented by G.R. Coope and M. Joachim (University of Birmingham) has the same interpretive difficulties. They examined fossil assemblages of beetles from sediments at St Bees, Cumbria, which just predate the Younger Dryas. They showed a sequence of species moving from those with present distributions of a southern European type (occurring before 12,000 yr ago) to those with present ranges restricted to northern Scandinavia (occurring after 11,600 yr ago). They point out that, although this suggests increasing coldness of climate, two facts make precise climatic interpretations difficult. First, there are no modern equivalents of these beetle communities and, second, in times of rapid climatic fluctuation, as these undoubtedly were, stable communities in which competitive sorting had taken place were never established.

The plant fossil record is equally subject to these problems. J.B. Macpherson (Memorial University of Newfoundland) attempted an analysis of Younger Dryas climates on the basis of certain indicator pollen types. At a kettlehole site in the Spey Valley, Scotland, she found a pollen sequence in Younger Dryas sediments which led from an *Empetrum* maximum, through an *Artemisia* maximum, back to a further *Empetrum* peak. Together with evidence from sediment stratification, she interprets this as indicating a climatic sequence of oceanic, high snowfall, through continental, low precipitation, back to oceanic once again.

*Empetrum* was long ago used as an indicator of oceanicity by Jessen (*Proc. R. Ir. Acad. B* 52, 85; 1949) and its value in this respect has been discussed at length by Brown (*New Phytol.* 70, 841; 1971). The argument is based entirely on the present range of the genus and experimental data are lacking. Its modern distribution is complicated because it is found in continental areas (for example, Siberia), where it occurs mainly under coniferous forest canopies. It survives in sites of low winter temperature if there is adequate snow-lie. Many species of *Artemisia* do

have more continental distributions, but it is a large and ecologically varied genus and the assignment of a blanket 'continental' label is undoubtedly an oversimplification. It is not impossible that the balance between *Empetrum* heath and *Artemisia* herb communities could be determined by a factor such as wind speed and frequency, which would affect snow-lie.

W. Pennington (University of Leicester) reported the results of a quest for present-day equivalents of the lost plant assemblages of the Younger Dryas. European sites have proved of limited value because of the current high latitude extension of forest due to the oceanic influence of the Gulf Stream drift. Greenland, on the other hand, has very restricted forest and hence is a more likely site for the existence of plant communities resembling those of the late-glacial in their pollen rain spectra.

The coastal fringe of western and southern Greenland has a low rainfall (100-250 mm) and, growing on bare patches of soil, *Artemisia borealis* is locally frequent. The surface lake sediments from such areas have up to 4 or 5% *Artemisia* pollen, which approximately corresponds to the percentage cover of the plant in the catchment area. These values are similar to those found in Younger Dryas pollen assemblages in northern Scotland.

A recently published pollen diagram from Loch of Winless in Caithness, North-East Scotland by Peglar (*New Phytol.* 82, 245; 1979) has about 2-3% *Artemisia* during the presumed Younger Dryas, which falls to zero with the commencement of the Holocene (Flandrian). At the transition there is a peak of more than 5% *Empetrum*. If this diagram is compared with that most recently published from North Wales, Melynlyn in Snowdonia by Walker (*New Phytol.* 81, 791; 1978), a very similar sequence is found. *Artemisia* attains about 5% in the Younger Dryas and there is an expansion of Ericales (undifferentiated) at the opening of the Holocene. Evidently the changes of climate occurring over a short period at that time were more profound than those climatic differences between the two sites resulting from geographic, spatial factors at any given time.

The conclusion one must draw from these examples is that we are still far from being able to make precise statements concerning the climate of the Younger Dryas on the basis of biological evidence. The use of indicator species, even if adequate autecological information is available, can be misleading unless taken in the context of total assemblages. And the interpretation of whole assemblages is frustrated by the lack of precise present-day equivalents. □

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# review article

## Meteorology of the Indian summer monsoon

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*The seasonally varying monsoon circulations associated with the annual heating and cooling of the Asian continent are the most important large-scale aspects of the general circulation of the atmosphere. The Indian summer monsoon affects the lives and the economies of many countries in Asia. The study of this phenomenon has been mainly limited to the Indian subcontinent whereas it takes place over the entire Indian Ocean. The lack of information over the marine region will be removed this year during the first Global Atmospheric Research Programme Global Experiment with the implementation of the Monsoon Experiment (MONEX) observational programme.*

THE term monsoon has been used to describe the biannual complete reversal in the prevailing wind flow in the lower atmosphere over the Indian Ocean. The reversal of the air flow is mainly a consequence of the distribution of land and ocean characterised by a large continent in its northern part and a large ocean in its southern part (the topography is also important). The monsoon (south-west during the summer and north-east during the winter according to the direction of low-level flow) is primarily caused by differential heating between the continental areas and the oceans as a result of the zenithal march of the Sun. This was recognised in 1628 when Edmund Halley proposed that the monsoons of South Asia result from differential heating of land and sea surfaces. In 1921, Simpson<sup>1</sup> supported this hypothesis but also emphasised the role of the Himalayas.

The term monsoon does not, however, only apply to the wind regimes prevailing over the Indian Ocean, it also describes the phenomenon in the tropical and equatorial regions of Africa and South Asia. The Asiatic summer monsoon is the best known monsoon region, probably owing to the associated rainfall. Here the term monsoon refers to the rains which fall during the June–September period, although mariners usually associate the monsoon with winds.

### The burst of the monsoon

As the Sun moves northwards bringing spring to the Northern Hemisphere, heat lows begin to form over the continental regions surrounding the Arabian Sea in May. A trough of low pressure forms and extends from Somalia northwards across Arabia and into the principal heat low located over Pakistan; this low-pressure belt extends northeastwards into central India. By the end of May, the heat lows have become well established and the south-west monsoon flow spreads northwards over the Arabian Sea, India and the Bay of Bengal. This change in the lower troposphere occurs quite abruptly and simultaneously over all the Indian Ocean: within a few days the intensity of trade winds of the Southern Hemisphere increases and the cross-equatorial flow becomes established<sup>2</sup>. The rainfall is not synchronous with the dynamical changes. In a rainfall orientated

definition of the monsoon, the south-west monsoon sets in during late April and early May in Burma. The rainbelt then advances northwestwards over a broad latitudinal area reaching the Coromandel coast towards the end of May and the Thar Desert in early July (Fig. 1). This rain belt can be associated with the northwards motion of the ITCZ located over the head of the Bay of Bengal during the winter months. The advance is unsteady and is not evenly paced. At Delhi, for instance, the average date of monsoon arrival is 2 July, but between 1901 and 1950 the actual dates ranged from 17 June to 20 July, with a standard deviation of 7.8 days. The rainfall is so sudden and spectacular that it is known as the burst of the monsoon.

The upper air circulation also undergoes an abrupt change in the entire Northern Hemisphere. At the end of May, an elongated warm belt appears between the Arabian Desert and southwestern China along 28° N in the mid-troposphere. The westerly southern jet moves downstream into China and dissipates; it is replaced by an easterly jet. This jet appears first over the Bay of Bengal and expands eastwards to the Philippines and westwards to Africa. The mid-tropospheric anticyclone established during the pre-monsoon season disappears in June and is replaced at about 80° E by the upper trough moving from the Bay of Bengal. Over northern India and nearby, two anticyclones come into existence in June; one over the Thar Desert

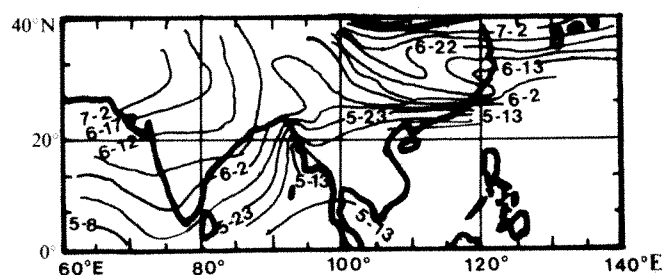
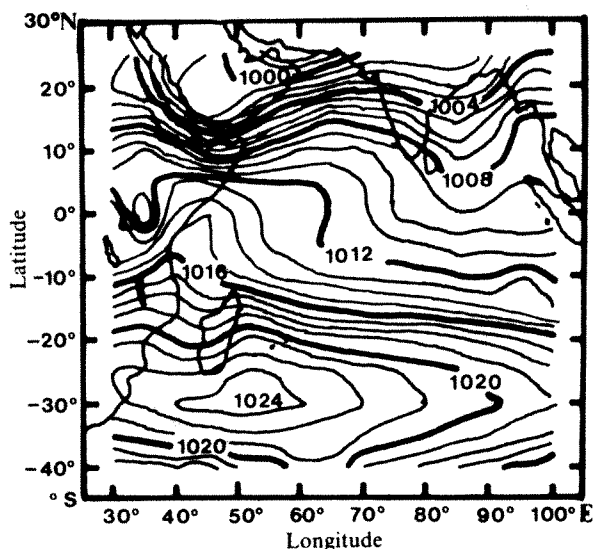


Fig. 1 Average onset dates of the rainy season (from ref. 57).



**Fig. 2** July mean sea level isobars (mbar) (from ref. 58). The mascarene high pressure over southern Indian Ocean, the monsoon trough over India and the equatorial trough to the east of 70° E can be seen.

(above 700 mbar) and another over southern Tibet (above 500 mbar).

These changes in circulation patterns that take place in June are many and varied, and meteorologists have tried to relate the changes to the burst of the monsoon. Some have related the advance of the monsoon with the shift of the upper trough and the westerly jet and the development of the easterly jet<sup>3</sup>. Other meteorologists think the burst takes place when the air from the Southern Hemisphere goes into the circulation over the subcontinent<sup>4</sup>. In fact there seems to be no cause-effect relationship between the development of the circulation in the lower and upper levels, the insolation and the influence of topography being prominent in both cases. Another closely related question is what determines the year-to-year variations in the date of the onset of the monsoon.

### Climatology of the Indian summer monsoon

As we have seen, certain elements are established over the Indian subcontinent during the pre-monsoon months. However, the Indian summer monsoon must be seen as a broad-scale system and it also includes components in the Southern Hemisphere. For a complete picture of the phenomenon all these elements must be described.

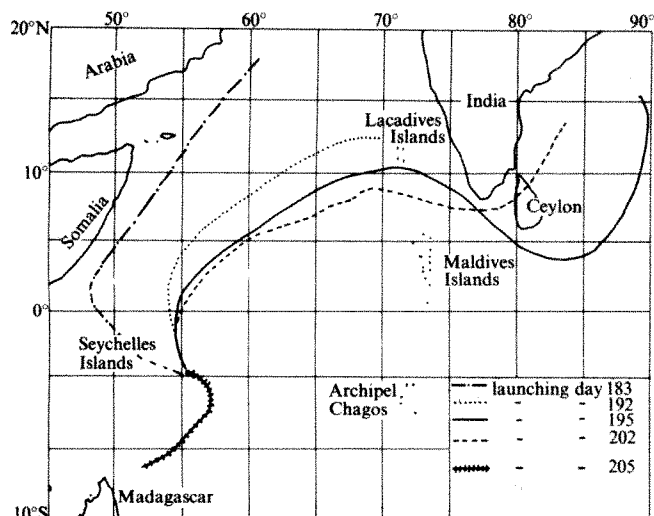
The monsoon trough over northern India is a very prominent feature of the synoptic weather charts during the summer season (Fig. 2). It is a part of the global equatorial trough of the northern summer season and indicates the separation between air of Northern and Southern Hemisphere origin<sup>5</sup>. It lies parallel to and about 450 km south-west of the Himalayas and is probably topographically anchored. The southwesterly to westerly air current from the Arabian Sea and the deflected air current from the Bay of Bengal meet along the Gangetic Valley giving rise to this trough which is connected to the Pakistan heat low. The topographical features of the subcontinent play an important combined dynamical and thermodynamical role in the location of the heat low and the development of the Gangetic Valley trough. The Himalayas force the monsoon current to ascend and thereby release latent heat. Thus, the mountains contribute significantly to making the monsoon circulation self-sustaining in the lower levels of the atmosphere and the Indian monsoon circulation different from monsoons in other parts of the world. The influence of topography on the streamflow also extends to higher levels. The heat low extends to above

850 mbar while the trough extends to about 500 mbar. Above 500 mbar the westerly and easterly flow is associated with the Tibetan anticyclone, to the north of 27° N, which has its maximum amplitude near 200 mbar.

Over the Arabian Sea the low-level flow from the Southern Hemisphere is the main characteristic of the monsoon. There has been controversy about the origin of the southwesterly winds<sup>6</sup> although recent experiments have demonstrated that these winds effectively correspond to the south-east trades of the Southern Hemisphere deflected after crossing the Equator, by the Coriolis force and the Southern Hemisphere trough located around 80° E on the Equator. This was shown by the measurements of radioactivity over the Arabian Sea which allows us to separate land and marine air<sup>7</sup> values. Another set of measurements was given by lagrangian trajectories of constant-level balloons flying in the boundary layer and launched from the Seychelle Islands (Fig. 3)<sup>8</sup>. The cross-equatorial flow is not uniform at all longitudes; it is weak in the eastern Indian Ocean where it enters the Bay of Bengal, and is strong along the East African coast (Fig. 4)<sup>9</sup>. Associated with the Southern Hemisphere trough on the Equator are weak westerlies found around 80° E and 5° S. Along the Somali coast, the air flow is characterised by one of the most intriguing phenomena of the monsoon: the low-level Somali jet which is in fact a major part of the southwesterly flow to the Indian continent (Fig. 5a and b)<sup>10</sup>. This low-level current, most pronounced at heights of 1–1.5 km, flows from the ocean near Mauritius over the northern tip of Madagascar to reach the Kenya coast at about 3° S. It then penetrates inland over the flat low-lying eastern areas of Kenya, Ethiopia and Somalia to reach the coast again near 9° N and then swings across the Arabian Sea to India. It splits into two parts over the sea, the more northern branch intersecting the west coast of India near 17° N while the southerly branch moves eastwards just south of India. This jet is occasionally reinforced or even temporarily replaced by a stream of air moving northwards up the Mozambique channel. The maintenance of the jet and its periodic fluctuations in strength are not well understood. A recent study using geostationary satellite images to determine the low-level air flow from cloud displacements<sup>11</sup>, suggests that the variation in strength (occurring with a quasi-biweekly period) result through the influence of cold fronts at mid-latitudes in the Southern Hemisphere which induce surges in the south-east trades as suggested earlier<sup>12</sup>. Its three-dimensional structure was studied in 1977 using an instrumented aircraft<sup>13</sup>. Jet-speed winds also occur over the central Arabian Sea and the Indian Peninsula in connection with the Somali jet<sup>14</sup>. The problem of the numerical simulation of this jet has been examined<sup>15</sup>. The  $\beta$ -effect, the orographic barriers over East Africa and Madagascar and a broad-scale monsoon forcing over India are some of the crucial elements for a proper simulation. Off the Somali coast, the atmospheric jet passes over the well known area of upwelling cold-coasted water which has also been numerically simulated<sup>16,17</sup>.

The air mass over the Arabian Sea consists of an inversion layer which exists over the flat lowlands of eastern Africa and over most parts of the Arabian Sea, inhibiting the development of deep clouds<sup>18</sup>. The height of the base of the inversion layer, which probably separates the low-level deflected moist trades from drier continental air above, increases eastwards and becomes less marked. The inversion layer disappears east of about 65° E where low-level convergence and ascent in the troposphere favour the dominance of deep clouds and occasional development of monsoon disturbances. How the Western Ghats affect the destruction of the inversion is important<sup>19</sup>. This inversion layer is a control factor in the air-mass modification over the Arabian Sea and can affect the efficiency of monsoon heat engine.

Another important feature of the monsoon system is the upper tropospheric tropical easterly jet near 150 mbar<sup>20,21</sup>. It has winds of roughly 80–100 knots and the strongest winds are found just to the west of the southern tip of India. Its intensity oscillates with a quasi-biweekly period (phase of generation and



**Fig. 3** Trajectories of constant-level balloons at 900 mbar, launched from the Seychelle Islands in 1975 (from ref. 8). Days are 1975 Julian days. The trajectory going towards Madagascar corresponds to a local reversal of the monsoonal flow due to the westward propagation of a tropical disturbance near 10° S (ref. 59).

degeneration early noticed by workers) and it must be seen as a part of the global upper air circulation in the tropics<sup>22</sup>. A numerical model of the broad-scale monsoon system with a few additions can simulate many of the well known broad features of the monsoon and particularly the easterly jet<sup>23</sup>.

It seems difficult to define limits of the monsoon circulation. Reversal of flow in zonal and meridional direction from lower to upper tropospheric has led some authors to suggest a closed circulation. The inflow and outflow from the monsoon area are linked through the general circulation in other areas. With the advent of summer, an east-west Walker circulation with ascent over north-east India and descent over the Pacific and a north-south meridional circulation with ascent over north India and descent over equatorial regions are set up<sup>24</sup>. The conditions in which the Walker circulation changes its intensity and location and the way in which it interacts with the monsoon circulation are largely unknown.

### Monsoon depressions

Monsoon depressions are an important component of Indian weather and have been studied for several years. About 1-3 depressions form per month during the monsoon months, particularly in July and August. These depressions begin to be seen over the Bay of Bengal where satellite photographs show cloud clusters. They do not acquire hurricane intensity during the summer months, because they are not long over the oceanic areas before landfall and the large-scale vertical wind shear over the troposphere inhibits local accumulation of the latent heat release over the disturbance. Heavy rainfall associated with these depressions is the most outstanding associated phenomenon. Up to 300-400 mm of rain in 24 hours have been recorded. The structure of these perturbations has been studied from observations made once the depression has moved over central India where some upper air observations are available<sup>24,25</sup>. The composite structure has also been studied<sup>27</sup>.

The depression is an intense low-pressure system with a vigorous associated circulation; winds of up to about 50 knots have been noted (Fig. 6). Whereas the typical horizontal scale of the depression is known to be around 500 km in the formative stage, it is typically 1,500 km over land. The disturbance has a cold core with its vertical axis tilting eastwards with a slightly warm core above. The maximum rainfall rates occur to the west of the depression. The perturbation is confined to the lower troposphere from the surface up to 400 mbar. The strongest

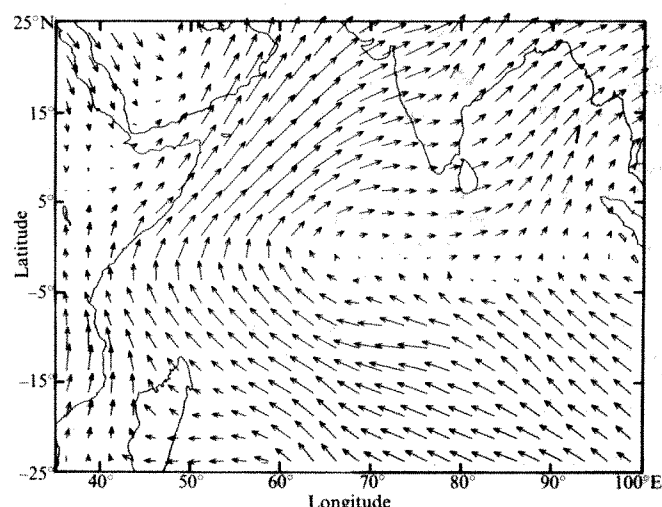
activity is concentrated in a narrow vertical tube ahead of the depression, characterised by strong cyclonic activity, convergence and upward motion. Relatively weak activity is found behind the depression where anticyclonic vorticity, divergence and subsidence are noted. Case studies indicate that the disturbance is imbedded in a region where the conditions necessary for the combined barotropic-baroclinic instability to exist are satisfied. Monsoon depression is primarily maintained by cumulus convection. The cumulus convective heating generates eddy-available potential energy by releasing heat at appropriate levels above the cold core. Here the rising of relatively warm air contributes significantly to the generation of eddy kinetic energy of the depression.

Whereas early studies suggested *in situ* formation in the Bay of Bengal<sup>28</sup>, recent analyses have led to a proposal of scale interactions of westward moving wavetrains. Such an interaction gives rise to a westward propagating downstream amplification<sup>29</sup>.

Mid-tropospheric cyclones are also an important part of the south-west monsoon. They can be found in the northeastern part of the Arabian Sea and in northern Bay of Bengal. They are known either to move very slowly westwards or remain quasi-stationary for many days<sup>30,31</sup>. The scale is of the order of about 3,000 km horizontally and 5 km vertically. Their largest amplitude is near the 600 mbar surface and they appear as a closed cyclonic circulation generally in the middle troposphere. They usually have a pronounced warm core above the middle level and a slightly cold core below that level. They are usually characterised by very intense convective and non-convective rainfall rates: total rainfall amounts of the order of 20 cm per 24 h can be noted. Flows near the surface and at the 200 mbar level do not show closed circulation. Despite case studies, detailed analysis of these depressions as well as numerical studies are necessary for a clear understanding of the development mechanism of the mid-tropospheric disturbances over the entire monsoon region.

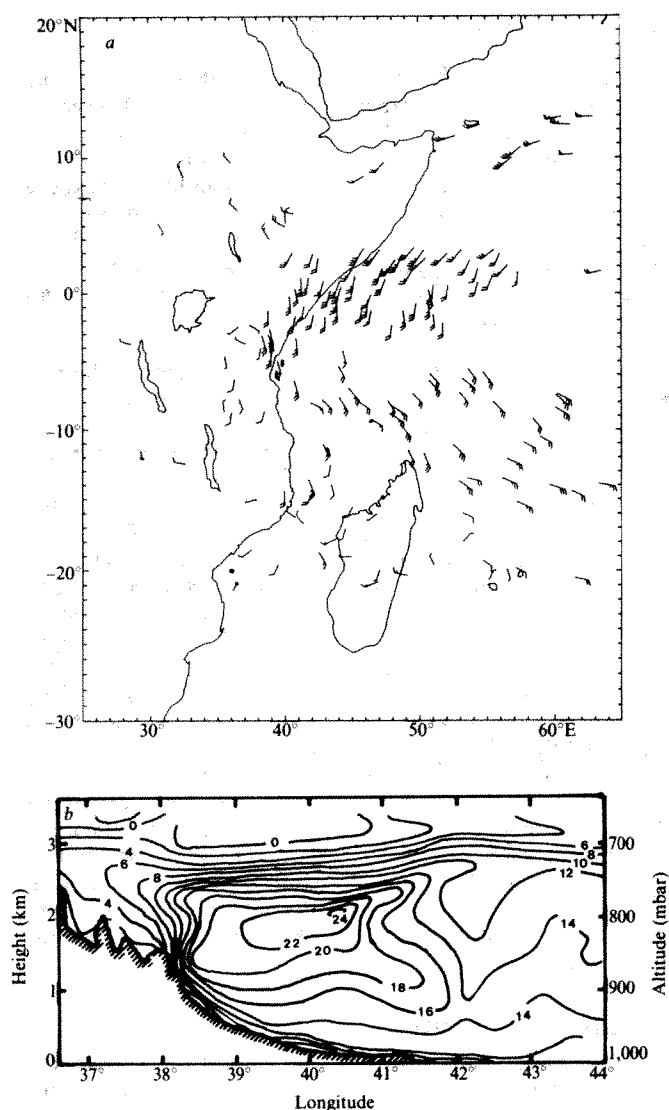
### The monsoon rains

A rapid build-up of atmospheric moisture over India augers the beginning of its monsoon season. Precipitable water vapour increases nearly twofold from March to June<sup>32</sup> and this high water-vapour content of the monsoon air is largely responsible for the heavy rainfall in the summer. The summer rainfall is the main component of the annual rainfall over the subcontinent except in Kashmir and the extreme south of the Peninsula and the east coast area: nearly 80% of India's total annual rainfall falls during the months June to September<sup>33</sup>. The orographic



**Fig. 4** Wind field over the Indian Ocean on 14 July 1975 as determined by an objective assimilation of ship reports (from Cadet and Reverdin, personal communication). The length of the arrow is proportional to wind intensity.





**Fig. 5** *a*, Low-level wind vectors determined from cloud displacements on 23 July 1978 inferred by METEOSAT pictures (from ref. 11); *b*, vertical and longitudinal structure of the meridional (southerly) wind component along the Equator on early morning of 4 July 1975 (from ref. 13).

influence for producing strong convergence in the lower layer is important in the distribution of rainfall. The west coast of India receiving the southwesterly flow and the Burma coast affected by the Bay of Bengal branch of the monsoon receives considerably more rain than the lee-side of the Western Ghats and Burma Mountains. The important rainfall over central India is largely due to the influence of the depressions moving from the north Bay of Bengal and to some extent due to convergence at the trough and the southwards slope of the trough axis with height. The only region contrasting with the common view of heavy rains is the Thar Desert in the western half of northern Indo-Pakistan. The presence of the air mass inversion between the lower shallow moist current (about 800 mbar) and upper drier air mass<sup>34</sup> causes less rainfall in this area.

The low-level current over the Arabian Sea originates in the Southern Hemisphere. However, the origin of moisture contained in this southwesterly flow is still questionable. A large part of the moisture available over the Indian subcontinent is supplied by the Arabian Sea branch of the monsoon. Over India the major water-vapour influx takes place across the Trivandrum-Bombay section, and the layer from the surface up to 700 mbar contributes 86% (ref. 35). The onshore fluxes are well correlated with rainfall along the west coast although a small

fraction is converted into precipitation (13%)<sup>36</sup>. While early estimations led to the conclusion that the contribution from cross-equatorial flow is less than that from the evaporation over the Arabian Sea, further computations have shown the existence of a certain water-vapour influx from the Southern Hemisphere<sup>37</sup>. Some of the flux (30%) into the Indian subcontinent comes from the Southern Hemisphere. The maximum cross-equatorial moisture flux takes place along the East African coast and can be associated with the low-level Somali jet. Observations suggest that some relationship exists between rainfall in India and the Somali jet: increased cross-equatorial flow followed by increased rainfall over western India<sup>10</sup>. However, this question deserves careful consideration<sup>38</sup>. Also, during a drought year the seasonal mean Somali jet is weaker than during a normal year<sup>22</sup>. An appreciable amount of moisture seemed to be picked up over the Arabian Sea. Thus, positive correlations have been found between sea-surface temperature over the Arabian Sea and monsoon rainfall over western and central India<sup>39</sup>. Tests, made with a general circulation model, suggest that rains over India are sensitive to change in sea surface temperature over the Arabian Sea<sup>40</sup>.

Monsoon rains exhibit considerable variations both from one year to the next and within one year (Fig. 7). Thus, the Indian summer monsoon has two basic synoptic patterns: the active and the break (or weak) monsoons. The succession of these patterns gives the well known pulsatory nature to the Indian monsoon which also manifests itself in the wind field. Thus, the monsoonal flow in the lower troposphere (below 500 mbar) undergoes rather drastic changes in association with rainfall variations: during active monsoon periods, a series of disturbances form and move west-northwestward across the Gangetic Plains and rainfall is widespread over the monsoon region. These interruptions, or breaks, last between 3 and 10 days (2–3 weeks in some years), and usually occur between the dissipation of one monsoon depression and the formation of another. During this phase of the monsoon, the monsoon trough shifts to the north from its normal position and heavy rainfall is concentrated along and near the foot of the Himalayas. Low-level westerlies shift north to the Gangetic Plains and in peninsular India they usually weaken and decrease in depth. The upper tropospheric easterlies spread to the north. During such epochs, when the monsoon trough is shifted to the foothills of the Himalayas, a secondary trough forms over the south Bay of Bengal and the south Arabian Sea near the Equator. Thus, there is a well marked negative correlation between the change in cloud density over central India and the equatorial Indian Ocean<sup>41</sup>. Feeble mid-tropospheric lows move from east to west in this trough. The monsoon can be revived under the influence of mid-tropospheric lows moving northwestwards along the west coast of India<sup>42</sup> but there may be other ways of initiating this monsoon sequence<sup>43–46</sup>.

Moisture distribution over the Arabian Sea is different during the two phases of the monsoon. During weak monsoon conditions, the moisture field is stratified whereas this stratification disappears during active monsoon and upwards transport of water vapour occurs. Evaporation seems to be larger during an active spell than during a weak one. During active monsoon, a part of the water vapour obtained by evaporation is transported towards the Indian subcontinent, the other being precipitated over the sea whereas during weak monsoon, the evaporation is used locally in the weather development<sup>47</sup>.

Numerical simulations show that cold sea-surface temperature over the west Arabian Sea leads to a drastic reduction of monsoon rainfall over India because the cold sea decreases the evaporation rate and increases the surface pressure leading to a reduction in the cross-equatorial flow and moisture flux downstream<sup>40</sup>. Thus analysis of a series of sea-surface temperatures over the Arabian Sea shows that during a weak monsoon there is a significant drop in sea-surface temperature over a large area compared with a period of strong monsoon<sup>48</sup>. A numerical simulation experiment of the Somali jet shows that the cross-equatorial flow in the west Arabian Sea intensifies the jet as a

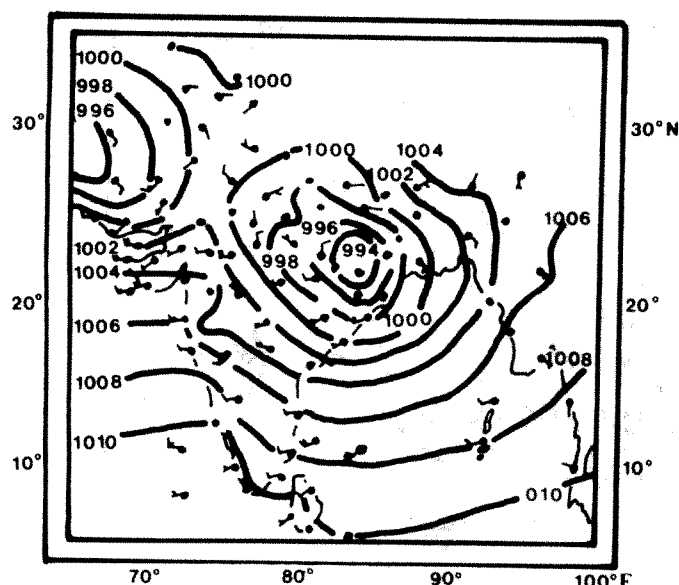


Fig. 6 Sea-level chart on 4 August, 1968 depicting a monsoon depression moving inland from the Bay of Bengal (from ref. 25).

result of the strengthening of the monsoon over India<sup>15</sup>. These and other experiments on the interaction between the Arabian Sea and the Indian monsoon suggest that the strength of the Indian monsoon does not depend on the strength of the cross-equatorial flow; this contrasts with other results that demonstrate a controlling influence of the Somali jet on the monsoon.

Active and break monsoons should be viewed as part of the fluctuations occurring on a very large scale and the changes in circulation of the Indian subcontinent might result from distant effects. Synoptic patterns have to be found which could characterise a break and an active monsoon<sup>49</sup>. The partition of the monsoon into two phases may be compared with the quasi-biweekly oscillation of the broad-scale monsoon elements. The two-week period may be divided into an active and a less active one and our study suggests an interesting ordering of the phase of the various elements of the monsoon system<sup>22</sup>. In fact, no unique or detailed description exists for active and break monsoon and numerical simulation may be useful for understanding the phenomenon and for separating the effects of the different synoptic components.

Long-term variability of monsoon rainfall has been studied in some detail by Indian workers. Quasi-biennial oscillation (QBO) in rainfall has been found at several stations in India<sup>50</sup> as well as in the annual rainfall in some meteorological subdivisions of the country and the monsoon rainfall as a whole<sup>51</sup>. Recent investigations using data of ISMEX-73 indicate that certain interactions exist between QBO and sea-surface temperature and monsoon currents in the equatorial area of the Indian

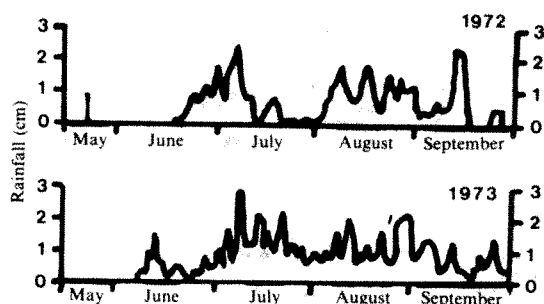


Fig. 7 Daily rainfall over central India (75°–85° E to 15°–25° N) during a drought year (1972) and a near-normal rainfall year (1973) (from ref. 22).

Ocean<sup>52</sup>. This result suggests that there is some interaction between the circulation in the tropical stratosphere which exhibits the well known QBO for zonal winds. There does not seem to be a significant relationship between Indian monsoon rain and solar activity<sup>51</sup>.

### The retreat of the monsoon

The monsoon in the Indian subcontinent begins to withdraw in mid-September when the circulation pattern in the Northern Hemisphere changes from a summer to a winter regime. The weakening of the easterlies, the southward shift of the monsoon trough and the appearance of the westerly jet stream in northern India are all reversals of the events that occur during the burst of the monsoon. In a year when a break takes place in September, the monsoon may retreat prematurely. The equatorward-motion of the ITCZ (inter-tropical convergence zone) brings abundant rainfall during the retreat of the monsoon<sup>53</sup>.

### Numerical simulation

The main objective of monsoon studies is to improve the ability to predict the atmospheric motion and associated rainfall over Asia. Empirical attempts have been made to predict, for example, the onset of the monsoon from wind activity over the Arabian Sea<sup>54</sup> or rainfall of western India from surges and lulls in the cross-equatorial flow<sup>55</sup>. Studies made to determine dynamical and thermodynamical structure of the atmosphere during break and active monsoon have been tentatively used to predict the occurrence of these different phases of the monsoon.

The development of numerical simulation is at an early stage but will be activated after the results of the Monsoon Experiment in 1979. Different types of numerical models can be developed. Specific regional fine-mesh models can be used to study certain components of the monsoon such as the Somali low-level jet, Bay of Bengal depression and mid-tropospheric disturbances. Global models with nested fine-grid system must be used to determine the conditions under which monsoon circulation interacts with the global circulation. Predictability experiments are urgently needed to investigate the feasibility of numerical weather prediction over the monsoon region for developing numerical prediction models.

### The experimental programme to study the Indian summer monsoon

For a long time, observations have been confined to the Indian Ocean although their deficiencies were recognised. The Indian Ocean is so large that international cooperation is necessary to make marine observations. An international experiment, the International Indian Ocean Expedition (IIOE), was conducted in 1963–64 and produced a large amount of information about the development of the monsoon over the Indian Ocean<sup>56</sup>.

The idea of a special monsoon experiment to be conducted during the First GARP Global Experiment (FGGE) was proposed in 1971. This idea was developed through two experiments: ISMEX-73 and MONEX-77. ISMEX-73, conducted by USSR and India, took place between 15 May and 10 July 1973. MONEX-77 took place during the summer of 1977 for the study of the Indian summer monsoon, and the winter of 1977 for the study of the winter monsoon over the Malaysia–Indonesia region. These two limited experiments considerably increased our knowledge of the development of the monsoon over the Indian Ocean and the structure of some monsoon elements (monsoon depressions and Somali low-level jet). They prepared the way for the main experiment to be conducted in 1979.

The main experiment, MONEX-79, has two components: the winter monsoon and the summer monsoon. The winter monsoon was conducted during the First Special Observing Period (SOP I) of FGGE (15 January–15 March). The summer monsoon will take place from May to September with an intensive period during SOP II of FGGE (May–June)<sup>57</sup>. The

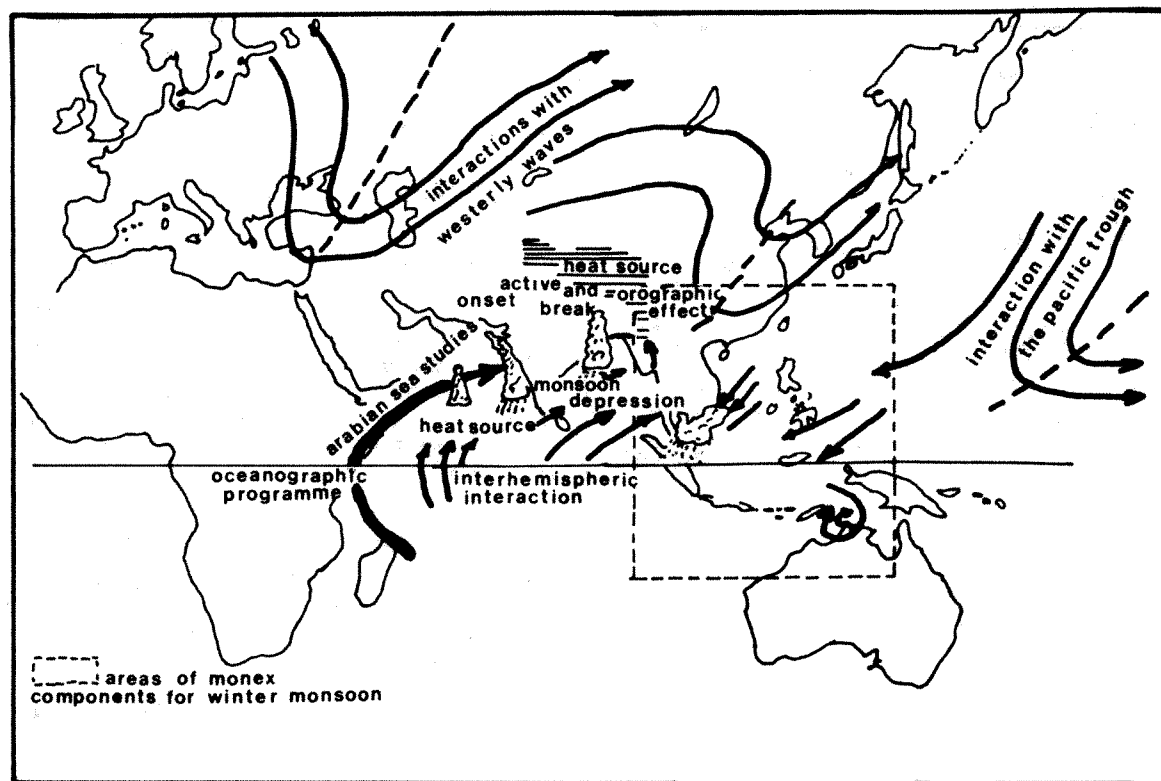


Fig. 8 MONEX-schematic illustration of major component programmes (from ref. 57).

summer MONEX can be divided into two phases. The first coincides with the SOP II and, taking advantage of the ship network over this area, will study the development of the monsoon over the Arabian Sea (Fig. 8). Instrumented US aircraft will study the structure of the atmosphere. About 90 superpressure constant-level balloons will be launched by a French group from the Seychelle Islands and Diego Suarez (Madagascar) to fly in the tropical boundary layer. The balloons tracked by the TIROS-N satellite will provide, along with their positions, recordings of pressure, temperature and moisture at flight level. This experiment will complement the boundary layer data by giving lagrangian trajectories of the mixed layer over the ocean. At the beginning of July the ships will move to the Bay of Bengal for the second period to study monsoon disturbances generated over this region. For the entire period (May to September), the surface synoptic network has been strengthened. Commercial aircraft and ships will enhance the network (especially the aircraft which will provide data on upper tropospheric winds over the ocean). The space-based part of the global observing system will be very important, especially the geostationary satellite over the Indian Ocean (GOES-1) from which winds can be determined by cloud tracers. Thus, with the high resolution geostationary satellite picture adequate low-level winds over the Indian Ocean can be deduced and a daily description of the circulation obtained. An important oceanographic programme will also be carried out during MONEX.

## Outlook

From the MONEX and FGGE data sets obtained in 1979, it will be possible to study the Indian summer monsoon on both a regional and a global scale. Undoubtedly the knowledge of the phenomenon will be improved within a few years. All this information is necessary for the development of reliable numerical models for weather forecasting. Such numerical forecasting of the vagaries of the Indian summer monsoon would benefit the populations of southern Asia. However,

forecasting needs continuous information about the present state of the atmosphere. The large marine extension of the phenomenon means this can only be made on an operational basis by space observatories; hence polar orbiting and geostationary satellites are needed.

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# articles

## How tidal heating in Io drives the galilean orbital resonance locks

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*Tidal heating in Io is the most likely energy source of its volcanic activity. Tidal dissipation in Io and Jupiter also controls the resonance configuration among the three inner satellites. The formation of the several resonance locks is described in detail in this article. This model sets limits on the Q values of both Io and Jupiter.*

THE Voyager 1 spacecraft fly-by of Jupiter provided startling images of the several active volcanoes on Io. Tidal heating in Io has been identified as a potentially important energy source<sup>1</sup> and it has been suggested that this same mechanism also controls the evolution of several orbital resonance locks among the three inner satellites. This article discusses in more detail how these resonance locks are maintained and describes the effects of dissipative tides in both Jupiter and Io on their establishment and evolution. The most significant results of this study are that the three-body resonance may have formed  $<5 \times 10^8$  yr ago and that a measurement of the rate of tidal heating in Io not only determines, say, the  $Q$  value of Io, but also  $Q_J$  of Jupiter. Arguments are also given which limit  $Q_J$ :  $2 \times 10^5 < Q_J < 2 \times 10^6$ . The factor  $Q/2\pi$  is equal to the ratio of the peak strain energy in each tidal flexing cycle to the energy dissipated per cycle<sup>6</sup>.

The most well known resonance involves the three inner galilean satellites, Io (1), Europa (2), and Ganymede (3). The mean longitudes  $\lambda$  of these three satellites when combined in the following combination:  $\lambda_1 - 3\lambda_2 + 2\lambda_3$  happens to equal an odd multiple of  $180^\circ$ . Lieske<sup>2</sup> found that the libration amplitude of this resonance variable is  $0.066^\circ \pm 0.013^\circ$  and that the period is 2,074 d. The other set of resonance variables involve the near commensurability of the mean motions of the inner pair and the forced precession of their pericentres,  $\tilde{\omega}_1$  and  $\tilde{\omega}_2$ . The mean daily motion of  $\tilde{\omega}_1$  and  $\tilde{\omega}_2$  is  $-0.7395^\circ \text{d}^{-1}$ . Sinclair<sup>3</sup> and Chao<sup>4</sup> noted that the arguments  $\lambda_1 - 2\lambda_2 + \tilde{\omega}_1$ ,  $\lambda_1 - 2\lambda_2 + \tilde{\omega}_2$  and  $\lambda_2 - 2\lambda_3 + \tilde{\omega}_2$  executed small amplitude librations about either 0 or  $180^\circ$ . In fact, it is just the interaction of the last pair of variables which predominately controls the three body couple.

Table 1 contains some of the pertinent physical data<sup>2,10</sup>: mass  $M$ , radius  $R$ , semi-major axis  $a$ , mean daily motion  $n$  and both free forced eccentricities. The free or proper eccentricity

represents that eccentricity which would remain if we could slowly remove all perturbing forces. The libration amplitude of the 2:1 resonance is equal to the ratio of the free to the forced eccentricity.

### Tidal interaction on Io

It is a common misconception that the principal tide on a synchronously rotating satellite moving in an eccentric orbit is radial. This idea can be traced to MacDonald's<sup>5</sup> purely phenomenological model which tried to incorporate the restriction that tidal dissipation in a synchronously locked satellite cannot transfer angular momentum from its spin to its orbit. In reality, the dominant tide moves back and forth across the mean sub-planet point as the satellite moves in its orbit (see Fig. 1). At pericentre, where dissipation and the resultant torque are greatest, the orbital angular motion is  $n(1+2e)$ . As seen from the planet, the satellite seems to rotate retrograde with angular velocity  $\sim -2en$ . Dissipation in the satellite causes a time delay ( $\approx 1/Qn$ ) of high tide leading to a displaced tidal bulge which leads the instantaneous sub-planet point. The torque by the planet acts to spin up the satellite but is prevented from doing so because of the existence of a permanent bulge. The effect of dissipation is to displace the rigid bulge until the resulting torque by the planet exactly cancels the average tidal torque.

The equations describing the evolution of the satellite orbit due to the inelastic planetary and satellite tides are<sup>6</sup>:

$$\frac{1}{n} \frac{dn}{dt} = -c(1 - (7D - 12.75)e^2) \quad (1)$$

**Table 1** Physical data of the three inner galilean satellites

	Io	Europa	Ganymede
$M/M_J \times 10^5$	$4.684 \pm 0.022$	$2.523 \pm 0.025$	$7.803 \pm 0.030$
$n$ (deg per day)	203.4890	101.1747	50.3176
$a$ (km)	422,000	671,400	1,071,000
$e_{\text{forced}} (2:1)$	0.0041	0.0101	0.0006
$e_{\text{free}}$	$1 \pm 2 \times 10^{-5}$	$9 \times 10^{-4}$	0.0015
$R$ (km)	$1,818 \pm 3$	$1,533 \pm 27$	$2,608 \pm 32$

$$\frac{de^2}{dt} = -\frac{2}{3}c(7D - 4.75)e^2 \quad (2)$$

$$D = 2k\left(\frac{R}{R_J}\right)^5 \left(\frac{M_J}{M}\right)^2 \frac{Q_J}{Q}; c_{10} = 6.1 \times 10^{-13} Q_J^{-1} \text{ s}^{-1} \quad (3)$$

For small homogeneous satellites the Love number  $k \approx 3/19 \rho g R \mu^{-1}$  where  $\rho$  is the density,  $g$  the surface gravity and  $\mu$  the rigidity. If Io were lunar-like then  $\mu \approx 6.5 \times 10^{11}$  and  $k = 0.027$ . Incidentally, Jupiter's fluid Love number equals 0.50. The parameter  $D$  is the ratio of the tidal scale factor of Io to that of Jupiter. For Io we find that  $D \approx 1,300$  if we adopt 'plausible' values for  $Q_J = 5 \times 10^5$  and  $Q_{10} = 100$ . We shall argue later that  $D \approx 4,300$ .

Suppose that Io were formed well inside the orbit of Europa about  $4.6 \times 10^9$  yr ago. Any initially free eccentricity in Io's orbit would be quickly damped by Jupiter, and Io's orbit would thereafter expand, driven by the dissipative tide it raises on Jupiter. Europa's orbit would also expand, but because of Io's greater mass and smaller semi-major axis, Io would spiral out faster. Io would eventually approach the 2:1 commensurability with Europa. This near resonant interaction is adequately described by the following set of differential equations<sup>4,7</sup>.

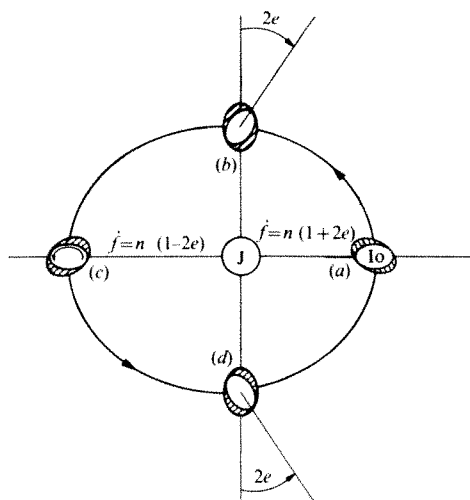
$$\frac{dn_1}{dt} = 3 \frac{M_2}{M_J} n_2^2 \left(\frac{a_2}{a_1}\right)^2 [e_1 C_{11} \sin(V_1 + \tilde{\omega}_1) + e_x C_{21} \sin(V_1 + \tilde{\omega}_2)] + \frac{dn_1}{dt_T} \quad (4)$$

$$\frac{dn_2}{dt} = -6 \frac{M_1}{M_J} n_2^2 [e_1 C_{12} \sin(V_1 + \tilde{\omega}_1) + e_2 C_{22} \sin(V_1 + \tilde{\omega}_2)] \quad (5)$$

$$\frac{dp_1}{dt} = -in_2 \left(\frac{a_2}{a_1}\right)^{1/2} \frac{M_2}{M_J} C_{11} \exp(iV_1) - \left(\frac{7}{3} Dc + i\dot{\omega}_{s1}\right) p_1 \quad (6)$$

$$\frac{dp_2}{dt} = -in_2 \frac{M_1}{M_J} C_{22} \exp(iV_1) - i\dot{\omega}_{s2} p_2 \quad (7)$$

where  $V_1 = \lambda_1 - 2\lambda_2$ . The coefficients  $C_{11} \approx C_{12} = C_1 = -1.18$  and  $C_{21} \approx C_{22} = C_2 = +0.42$  are polynomials in  $a_1/a_2$ . The variations of the pericentre  $\tilde{\omega}$  and eccentricity are most easily described by the variables  $p = e \exp(-i\tilde{\omega})$  and  $q = p^*$ . The parameter  $\dot{\omega}_s$  is the mean precession rate of the orbital pericentre due to the jovian oblateness, satellite interactions, and so on. For Io,  $\dot{\omega}_s = +0.16^\circ \text{ d}^{-1}$ . This term and the tidal acceleration  $f$  Europa shall be ignored in the following discussion—Europa's acceleration is unimportant because the coefficient  $c_2$  is small ( $\approx 0.021c_1$ ) while the ratio  $D_2 \approx 1,000$ .



**Fig. 1** Location of tidal bulge of a synchronously rotating satellite at different points in its orbit. The tide lags behind at pericentre (a) where the orbital angular velocity  $f$  is greatest. The optical libration of the permanent bulge has the opposite phase and is  $2e$  radians behind a point (b) and ahead at point (c).

A solution for the forced motion of  $p$  can be obtained by assuming that  $\dot{V}_1 = \nu_1$  is constant. This approximation is valid as long as either  $\nu/n$  is large compared to  $(eM_1/M_J)^{1/2}$  or the free eccentricity is small compared to the forced eccentricity, both of which happen to hold in this case. We find

$$p_1 = -\frac{M_2}{M_J} \frac{n_2}{\nu_1} \left(\frac{a_2}{a_1}\right)^{1/2} C_1 \exp(iV_1 + \delta) \quad (8)$$

$$p_2 = -\frac{M_1}{M_J} \frac{n_2}{\nu_1} C_2 \exp(iV_1) \quad (9)$$

The mutual perturbation leads to a forced retrograde motion of the pericentres of both orbits. Dissipation in Io induces a phase lag  $\delta = \frac{7}{3} Dc/\nu_1$  in Io's pericentre. This phase lag means that Io is slightly closer to Europa E just after than just before conjunction (see Fig. 2). Thus the torque, when averaged over the conjunction phase, tends to accelerate Europa in its orbit and decelerate Io.

Substituting the above solutions for  $p_1$  and  $p_2$  into the equations (4) and (5) for  $\dot{n}_1$  and  $\dot{n}_2$ , we obtain

$$\frac{dn_1}{dt} = 7cn_1 D e_1^2 + \frac{dn_1}{dt_T} = -cn_1(1 - 14De_1^2) \quad (10)$$

$$\frac{dn_2}{dt} = 14cn_1 D \left(\frac{a_1}{a_2}\right)^2 \frac{M_1}{M_2} e_1^2 \approx -10.3cn_1 D e_1^2 \quad (11)$$

As Io tidally evolves outwards, Io's forced eccentricity increases until it reaches the critical value  $\approx 1/(35D)^{1/2} \sim 0.0026$ . Europa's limiting eccentricity is  $\sim 0.0014$ . Thereafter, the relative outward acceleration of Europa causes its mean motion to be exactly half that of Io. This stable state is maintained until Europa encounters the 2:1 commensurability with Ganymede. The 4:1 commensurability of Io with Ganymede is third order in the eccentricities and represents a considerably weaker interaction. The inelastic tide raised on Europa will repel Ganymede. Still, the acceleration of Europa by Io is so great that Europa's forced eccentricity would have to be at least three times larger than its present value (0.0101) for this mechanism to push Ganymede out so that  $\dot{n}_2 = 2\dot{n}_3$ . Before Europa's eccentricity can be pumped up to this value the 2:1 frequency  $\nu_2 = n_2 - 2n_3$  of the outer pair approaches that of the inner pair,  $\nu_1 = n_1 - 2n_2$ . The vanishing of the difference frequency describes the presently observed three-body resonance.

## Coupling between Europa and Ganymede

The near resonant gravitational couple between Europa and Ganymede can be obtained by indexing the subscripts of (4-7) by one. The resulting distortion in the shape of Europa's orbit by both Io and Ganymede is

$$p_2 = -\frac{M_1}{M_J} \frac{n_2}{\nu_1} C_1 \exp(iV_1) - \frac{M_3}{M_J} \frac{n_2}{\nu_2} \frac{a_2}{a_3} C_2 \exp(iV_2) \quad (12)$$

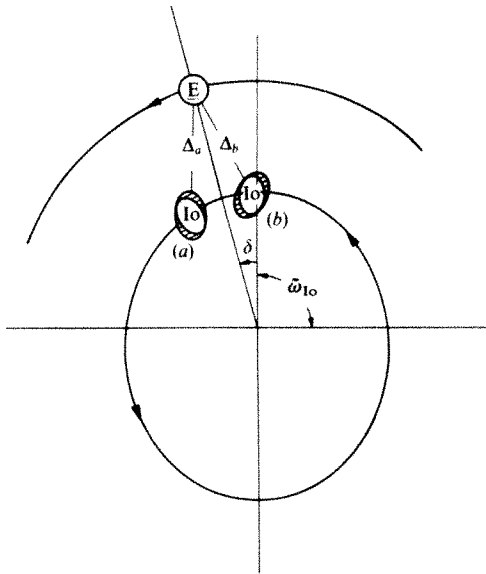
When this solution is substituted back into the differential equation for  $n_1$ ,  $n_2$  and  $n_3$ , we obtain the interaction involving the resonance argument  $\phi = V_1 - V_2$ .

$$\frac{d^2\phi}{dt^2} + An_2^2 \sin \phi + cn_1(1 - 45.0De_1^2) = 0 \quad (13)$$

$$\frac{d\nu_1}{dt} + 0.68An_2^2 \sin \phi + cn_1(1 - 34.6De_1^2) = 0 \quad (14)$$

The coefficient  $An_2^2 \approx -0.03858 (1 + 0.19 \cos \phi) (^\circ \text{ d}^{-1})^2$  if we include contributions from higher order effects (J. Lieske, personal communication).

The secular acceleration of  $\phi$  and  $V_1$  vanish when Io's forced eccentricity  $\approx 1/(13D)^{1/2}$ . As this eccentricity is 1.5 times larger than that necessary to maintain the 2:1 Io-Europa lock, this implies that the system passes through the capture phase (that is, when  $\dot{\phi}$  vanishes and reverses sign) with the smaller eccentricity. After the three body lock is established, Io's eccentricity is pumped up until the quasi-stationary state is achieved.



**Fig. 2** Relative location of Io-Europa at equal time intervals before (b) and after (a) conjunction. Because of the tidal offset  $\delta$  in Io's pericentre,  $\Delta_b > \Delta_a$  and the average tangential torque repels Io from Europa.

The torque acting to repel Ganymede results from an offset of the resonant argument  $\phi$  from  $180^\circ$ . Actually, the transfer of momentum from Europa to Ganymede involves the same kind of mechanism outlined for the 2:1 case, except that in this case the offset in the forced motion of Europa's pericentre by Ganymede is coupled directly to the offset driving the Io-Europa pair. The next problem to resolve is the capture mechanism.

One mechanism for changing the energy and the libration amplitude of a pendulum-like equation is through the addition of a  $\dot{\phi}$  term. The dominant  $\dot{\phi}$  term in this case comes from the resonance-induced variation of Io's forced eccentricity which is inversely proportional to  $\nu_1 = n_1 - 2n_2$ . From analysis of the equations of motion (4-7, 13, 14) we find that near the three-body resonance the variations are  $\delta n_1 \approx 0.125\dot{\phi}$ ,  $\delta n_2 = -0.276\dot{\phi}$  and  $\delta n_3 = 0.023\dot{\phi}$ . The additional term to be added to the left hand side of equation (13) is therefore

$$+60.8cDe_1^2 n_1 \nu_1^{-1} \dot{\phi} \quad (15)$$

The coefficient of  $\dot{\phi}$  is positive, implying that the librational energy is reduced through this term. The resulting differential equation describing the motion and tidal evolution of the three-body resonance happens to be nearly identical to that of spin-orbit resonance<sup>8,9</sup>. An estimate of the probability that capture into resonance occurs can be obtained from comparing the change in the kinetic energy during the cycle that  $\dot{\phi}$  reverses sign to the maximum change per cycle. The resulting capture probability  $P_c$  is given by

$$P_c = \frac{2}{1 + \pi/4 [1 - 45De_1^2 / 60.8De_1^2 \nu_1^{-1} A^{1/2} n_2]} \quad (16)$$

Evaluating this expression with the appropriate values of  $e_1$ ,  $\nu_1$  and  $A$  at capture we find  $P_c \approx 0.90$ . Capture into the three-body resonance is almost certain.

The damping of the libration amplitude  $\phi_m$  is approximately described by<sup>9</sup>

$$2 \sin \frac{1}{2} \phi_m \approx \left( \frac{16}{\pi} \right)^{1/2} \left( \frac{A(0)}{A(t)} \right)^{1/4} \exp \left( -\frac{1}{2} \int \sigma(t) dt \right) \quad (17)$$

where  $\sigma = 60.8cDe_1^2 n_1 \nu_1^{-1}$ . Because forced  $e$  increases from  $1/(35D)^{1/2}$  to  $1/(13D)^{1/2}$  the coefficient  $\sigma$  varies from  $\sim 250c$  up to  $1,100c$ . The secular decrease of  $\nu_1$  can be obtained from averaging equations (13) and (14). The result is

$$\frac{d\nu_1}{dt} = -0.32cn_1(1 - 12.5De_1^2) \quad (18)$$

Given that  $\phi_m = 1.1 \times 10^{-3}$  ( $0.066^\circ$ ), we find that the time since capture  $\Delta t \approx 0.024c^{-1} = 1,200 Q_1$  yr. Furthermore, the relative change in Io's semi-major axis is only 0.7%. On the other hand, the present forced eccentricity  $\approx 1/(13.8D)^{1/2}$  is very near the limiting value of  $1/(12.5D)^{1/2}$ . From this we can deduce that  $D \approx 4,300$ . Limits on  $Q_1$  can be obtained from a comparison of the rate of tidal heating to that expected from radiogenic sources and an estimate of the total heat input during the lifetime of the three-body resonance.

From equating the rate of change in Io's orbital energy by the satellite tide to the tidal heating rate  $dE_1/dt$ , we obtain

$$\frac{dE_1}{dt} = \frac{1}{3} M_1 n_1 a_1^2 \frac{dn_1}{dt} \left( \frac{\text{Io}}{\text{tide}} \right) = \frac{7}{3} M_1 n_1^2 a_1^2 c D e_1^2 \quad (19)$$

The resulting estimate for the rate of heating is  $3.0 \times 10^{25} Q_1^{-1} \text{ erg s}^{-1}$ . This is to be compared with the radiogenic value of  $5 \times 10^{18} \text{ erg s}^{-1}$  for a body of Earth-like composition. The apparent fact that Io is much more active than the Earth suggests that  $Q_1 \ll 2 \times 10^6$ , if we assume that for a given heat input that surface activity scales with radius.

The total heat deposited in Io per gramme of material during the three-body resonance lifetime is only  $0.9 \times 10^{10} \text{ erg g}^{-1}$  or  $260 \text{ cal g}^{-1}$ . If no heat is lost, the mean temperature rise is  $\sim 900 \text{ K}$ , which is enough to partially melt Io.

A lower bound of  $Q_1$  of  $\sim 2 \times 10^5$  and an upper bound of the heat input in Io of  $\sim 10^4 \text{ cal g}^{-1}$  can be obtained from the condition that Io has significantly tidally evolved and that the 2:1 resonance lifetime is the age of the Solar System. The tidal heating of Europa is at most  $\sim 100 \text{ cal g}^{-1}$  because of its very small eccentricity ( $\approx 0.0014$ ) during the 2:1 resonance lifetime.

Voyager 1 images of Europa (fly-by distance  $\sim 750,000 \text{ km}$ ) revealed long linear features that may be due to tidally induced fracturing. The relative age of these features may be deduced from the much more detailed Voyager 2 images of Europa (fly-by distance  $\sim 190,000 \text{ km}$ ; resolution  $\sim 5 \text{ km}$ ). If indeed these features are of tidal origin, then they should have been produced in the past  $10^3 Q_1$  yr during which Europa's forced  $e$  has increased from 0.0014 to 0.0101.

It should be emphasised that all of the above conclusions are predicated on Lieske's solution for the libration amplitude and, of course, the story changes if this measurement happens to be spurious. The major change is that the three-body resonance may be very old. The lower bound on  $Q_1$  is then reduced to  $\sim 8 \times 10^4$  while the heat deposited in Europa could be as high as  $10^4 \text{ cal g}^{-1}$  because of its much larger forced  $e$  once the three-body resonance is established.

In the expansion of the Sun-satellite gravitational interaction, a forcing term exists with argument  $2\lambda_1 - \Omega_3 - \psi$  and a period of 2,076 d which is very near the libration period of  $2,074 \pm 10 \text{ d}$  (J. Lieske, personal communication).  $\Omega_3$  refers to the node of Ganymede's orbit and  $\psi$  to the node of Jupiter's equator to the jovian orbit. The amplitude of the forcing term is very sensitive to the difference in these periods. The determination of whether this observed libration amplitude is in reality due to this forced term rests primarily in improving the masses of the galilean satellites.

On the other hand, a more sophisticated tidal analysis which includes the third harmonic tides on Jupiter, europian tides and higher order contributions to the coefficient of the  $\dot{\phi}$  term are not expected to change the primary conclusion that the three body was established recently nor alter the above numerical estimates by more than  $\sim 20\%$ .

There are other observables which can be used to estimate the rate of dissipation in Io. Since Io is about the same distance from Jupiter as the Moon from the Earth, the maximum recession of Io should be no more than that found for the Moon ( $\sim 3 \text{ cm yr}^{-1}$ ) and is completely negligible. On the other hand, the variation in Io's longitude times its semi-major axis is quadratic in time. For a rate of  $1.5 \text{ cm yr}^{-1}$ , the magnitude is  $150 \text{ km}$  ( $\Delta t^2$  in cent.) and might be detectable using  $\sim 100$  yr old eclipse data.



Other possibilities are polar wander (thought to be driven by mantle convection on Earth) and a slightly faster than synchronous rotation. This latter possibility occurs if the non-hydrostatic component of the moment of inertia difference  $(B - A)/C$  is small compared with the average, periodic tidal component. The minimum time scale for either effect is set by the relaxation time  $\sim \eta h / \rho g R^2$  of the zero frequency tidal bulge. If the viscosity  $\eta \approx 10^{22}$  St (like the Earth's asthenosphere) and the crustal thickness  $h \sim 100$  km, this time scale is  $\sim 100$  yr. Thus these effects are unlikely to be observable from image analysis of the Voyager 1 and 2 fly-bys which are separated by only 154 d.

### Other implications

Some variants of this sequence of events can be dismissed because of their low probability. It is unlikely that Io first encounters the three-body resonance well before the 2:1 near commensurabilities are achieved, because the capture probability, which scales like  $\nu^{-7/2}$ , would be small. The possibility that Europa might have tidally evolved into a quasi-stationary 2:1 commensurability with Ganymede first limits the heat input in Io to  $\sim 200 \text{ cal g}^{-1}$ . This amount of heat is probably insufficient to drive the observed surface activity.

Another variation is that Io's forced eccentricity, when Io encountered either the 2:1 or three-body resonance, may have been pumped up significantly larger than its present value. This would be expected if Io were initially more rigid than at present because of the absence of a large fluid core. The nearly elastic periodic tides would rapidly build up until the elastic limit is reached after which massive satellite-wide fracturing occurs. The catastrophic increase in tidal dissipation and perhaps tide height could provide the trigger for core formation.

Among the other satellite resonance locks, only the saturnian pair Enceladus-Dione has an *e*-type lock where the free eccentricity of one member is exceptionally small. The forced eccentricity of Enceladus is 0.0044 while the free eccentricity is  $\sim 1 \times 10^{-4}$ . Enceladus is apparently a small icy satellite ( $M = 1.5 \times 10^{-7} M_S$ ,  $R \approx 270$  km and  $\rho \sim 1.0 \text{ g cm}^{-3}$ ). The parameter  $D \sim 60$  if we assume  $\mu \approx \mu_{\text{ice}} \sim 9 \times 10^{10}$  and  $Q_s/Q_{\text{En}} = 10^3$ . Thus it is unlikely that this pair has reached a steady state configuration. It is possible that originally  $e_{\text{En}}$  was pumped up to some critical eccentricity which caused catastrophic fracturing. The effective rigidity may have been sufficiently reduced, such that this pair too has achieved a quasi-stationary orbital configuration. The Voyager 2 fly-by of Enceladus may reveal another curious satellite whose past and present state is controlled by tidal friction.

### Conclusions

We conclude that tidal dissipation in Io is a plausible energy source for the volcanic activity and is the controlling mechanism which leads to the establishment and the present state of the several resonance locks. The most plausible sequence of events is in four stages. (1) All three satellites are in orbits far from either the 2:1 commensurabilities or the three body lock. The tide raised on Io quickly damps down the free eccentricity on a time scale  $\leq 10^5 Q_1$  yr. Only modest tidal heating in Io occurs. (2) The dissipative tide raised on Jupiter by Io is dominant and causes Io's orbit to spiral outwards. No significant tidal heating occurs. Io approaches the 2:1 commensurability with Europa in stage (3). Io's forced eccentricity rapidly increases to the critical value  $\sim 1/(35D)^{1/2} \sim 0.0026$ . Thereafter the resonant interaction causes Europa's orbit to expand at exactly half that of Io's orbit. Tidal heating probably leads to the formation of a fluid core. Finally (4) Europa approaches the 2:1 commensurability with Ganymede but instead of dissipation in Europa repelling Ganymede we find that Io must work even harder using the three-body resonance to transfer angular momentum from Europa to Ganymede's orbit. We again rapidly reach a steady state as  $e_{\text{Io}}$  approaches  $1/(13D)^{1/2}$ .

Based on the observed three-body resonance amplitude of  $0.066^\circ$ , we have concluded that the three-body resonance was established  $1,200 Q_1$  yr ago. About  $220 \text{ cal g}^{-1}$  could have been deposited by tidal heating during this interval and is just sufficient to produce a large fluid core in Io. The 2:1 near commensurability of Io may be considerably older than the three body lock. Upper and lower limits of  $Q_1$  are  $2 \times 10^6$  and  $2 \times 10^5$ . Analysis of Voyager data should provide an estimate of the energy necessary to drive Io's volcanic activity and provide a firm estimate of the jovian  $Q_1$ .

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## A twin-jet model for radio trails

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*Radio trails are interpreted in terms of the twin-jet theory of double radio sources. A simple dynamical model of a narrow jet bending under the ram pressure of the intra-cluster medium is calculated and applied to some observations of the source associated with NGC1265. Observed radio emission must come from the interior of the jet, which is surprisingly stable to disruptive instabilities.*

RADIO TRAILS (head-tail sources, twin-tail sources) are a distinctive morphological class of weak extragalactic radio sources<sup>1,2</sup>. The most natural interpretation of their radio shape is that the associated active elliptical galaxy, which would otherwise have produced a normal double source, belongs to a cluster and is moving at  $\geq 1,000 \text{ km s}^{-1}$  through an intracluster gas<sup>1</sup>. The radio-emitting regions are then swept backwards by ram pressure. The wide-angle trails (bent doubles<sup>2</sup>) such as 3C465<sup>3,4</sup> are an intermediate category where the motion of the galaxy

causes distortion to a lesser degree. The two best studied radio trails are 3C83.1B<sup>5-8</sup>, associated with the galaxy NGC1265 in the Perseus cluster, and 3C129<sup>5,6</sup>.

In many weak radio sources, there is now good morphological evidence<sup>2,9,10</sup> for continuous energy supply, via collimated jets or beams emanating from the nucleus of the associated galaxy or quasar<sup>11-13</sup>. Recently, Owen *et al.*<sup>8</sup> have produced a high resolution map of NGC1265 in which narrow, quasi-continuous streams can be seen emerging from the nucleus of a galaxy associated with a radio trail. These streams have a transverse angular scale  $\leq 2''$  and appear to be bent back into a curved arc until, at a distance  $\sim 30$  kpc from the nucleus (assuming a distance to the cluster of 100 Mpc), the jets disappear in high surface-brightness features and presumably merge into the trail. The radio emission from the beam at  $\leq 5$  GHz is  $\sim 10^{40}$  erg s<sup>-1</sup>, only a few per cent of the radio luminosity of the tail.

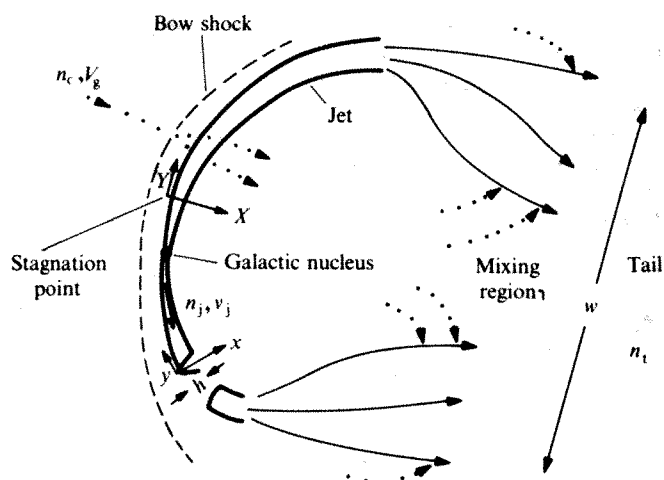
As argued by Owen *et al.*<sup>8</sup> these observations pose difficulties for the 'discrete blob' model of radio sources<sup>14-16</sup> in which independent gas clouds plough through previously undisturbed intergalactic medium. The distinctive feature of a jet interpretation is that each element of gas moves along a pre-established track (whose curvature is determined by the mean momentum discharge averaged over the recent history of the source) rather than on a trajectory depending on its individual cross-section and inertia. In NGC1265, the fact that there is a bright unresolved nuclear source indicates continuing activity and outflow from the galactic centre.

Here we give a simple discussion of the dynamics and equilibrium of a curved jet, assuming that the jet carries a steady flux of energy and momentum, and ignoring all complexities stemming from instabilities, entrainment, and so on. Then we make some quantitative estimates for NGC1265, and discuss the relationship between the curved jet and the extended tail. Further implications and various difficulties are also discussed.

## Dynamics of a curved jet

Consider a narrow, collimated, supersonic jet emerging from the central region. It must come into dynamical equilibrium with the pressure of the cluster gas sweeping past the jet. The flow pattern that we envisage is shown in Fig. 1. The intracluster medium (ICM) of electron density  $n_c$ , temperature  $T_c$ , flows towards the jet with the galaxy's velocity with respect to the centre of the cluster,  $V_g$ . We ignore gas motion in the ICM and assume that there is no significant mixing between the jet and the ICM, justifying this assumption *a posteriori*. If the galaxy moves supersonically, there will be a cylindrical stand-off bow shock; the stagnation pressure at the origin, where the jet is perpendicular to  $V_g$ , will exceed the ambient ICM pressure by  $p_0 = 2.9 M_g^2 n_c k T_g$  where  $M_g$  is the Mach number associated with the galaxy's motion through the ICM<sup>17</sup>. (This formula is also approximately valid for subsonic motion). The jet will be accelerated transversely within this transition region and the density profile will adjust to establish a pressure gradient across the jet, and to come into pressure equilibrium with the ICM either by expanding through a series of rarefaction waves or through being heated by a combination of weak shocks and compression waves. Downstream from the jet, the flow will come into rough pressure equilibrium with the ambient ICM and so the pressure difference across the jet will be roughly the same as  $p_0$ .

The rate of bending of the jet is governed by the jet density profile. In order to calculate a simple model, we treat the jet velocity  $V_j$  as constant, and the jet fluid as having a temperature  $T_j$  which depends on distance along the jet. We approximate the complex gas flow by relating the pressure at the jet surface to the stagnation pressure by the factor  $\cos^2 \phi$ , where  $\phi$  is the angle between the surface normal and the surface normal at the stagnation point<sup>18</sup> (see Fig. 1). This relates the pressure to the momentum flux incident on the surface in the shocked ICM. In cross-section, the jet profile then has the form  $y = 2h \cos^{-1} [\exp(-x/2h)]$  where the scale height is  $h = 2kT_j/m_p g$ ,



**Fig. 1** Schematic diagram of the bending of a jet by a transverse supersonic flow. Axes  $X, Y$  describe the equilibrium shape of the jet in a frame moving with the galaxy. Axes  $x, y$  describe the profile of the jet in cross-section. The pressure difference across the jet is comparable with the ram pressure of the intracluster medium. In the jet, this is opposed by the centrifugal force associated with the curved trajectory. After bending through a large angle, the jets disrupt and share their mass, momentum and energy fluxes with the shocked intracluster medium. Dissipation of relative kinetic energy creates a mixing region of transverse size  $\sim w$ . The mixture of thermal gas, relativistic particles and magnetic field flows downstream away from the galaxy to form the tail.

where  $g$  is the effective acceleration felt by the material in the jet as it follows its curved path.

We make a similar assumption about the variation of the pressure  $p$  along the jet axis. In a frame such that  $X$  and  $Y$  are the coordinates of the jet relative to the point where the velocity is perpendicular to the galaxy's velocity, the jet curvature is  $Y''(1 + Y'^2)^{-3/2}$ . For a given effective specific heat ratio  $\gamma$  in the jet we can calculate the trajectory from

$$g = V_j^2 Y''(1 + Y'^2)^{-3/2} = \frac{p_0}{n_{j0} m_p h_0} (1 + Y'^2)^{(1/2)\gamma - 1} \quad (1)$$

The stagnation pressure, jet electron density and scale height are  $p_0$ ,  $n_{j0}$  and  $h_0$  respectively. The scale height varies along the jet as  $h = (1 + Y'^2)^{1/2\gamma} h_0$ . From equation (1) we see that the jet Mach number  $M_j$  is related to the radius of curvature  $R_0$  at the stagnation point by<sup>19</sup>

$$M_j = (R_0 / \gamma h_0)^{1/2} \quad (2)$$

Note that if  $n_c$  and  $V_g$  are known, and  $h$  can be estimated from the radio contours, then the momentum flowing along the jet,  $\pi n h_0 R_0 p_0$ , can be inferred from the observations (apart from projection effects). The energy flowing along the jet is larger by the unknown factor  $V_j/2$ .

## Application to NGC1265 (3C83.1B)

The galaxy NGC1265 has a radial velocity  $\sim 2,300$  km s<sup>-1</sup> relative to the mean velocity of the Perseus cluster. Then

$$V_g \sim 2,300 \text{ sec } i \text{ km s}^{-1}; \\ M_g \sim 2.1 (T/5 \times 10^7 \text{ K})^{1/2} \text{ sec } i \quad (3)$$

where  $i$  is the angle between the galaxy's velocity (relative to the cluster centre) and our line of sight and  $T$  is the temperature of the ICM. If NGC1265 is bound to the Perseus cluster, then  $\cos i \geq 0.6$ .  $n_c$  is probably  $\sim 3 \times 10^{-4}$  cm<sup>-3</sup>; the X-ray data suggest  $T_c \sim 5 \times 10^7$  K, implying that  $M_g$  lies between 2 and 4, and vindicating our assumption of supersonic flow.

The internal pressure within the beam at the stagnation point is

$$p_0 \approx 3 \times 10^{-11} (n_c / 3 \times 10^{-4} \text{ cm}^{-3}) \text{ sec}^2 i \text{ dyn cm}^{-2} \quad (4)$$

An important check of the self-consistency of this model for NGC1265 is then to ask whether this value of  $p_0$  permits a high enough volume emissivity to account for the observed radio emission. This emission is non-uniform along the jet, the mean value being  $\sim 5 \times 10^{-12}$  dyn cm $^{-2}$ . This value is calculated on the assumption that the spectrum extends, with spectral index 0.8, up to 10 GHz (see ref. 8), but making no allowance for relativistic protons. In the individual 'hot spots' the pressure is higher; the precise value depends on the detailed geometry, but an estimate would be  $\geq 4 \times 10^{-11}$  dyn cm $^{-2}$ . The corresponding equipartition field is in the range  $(1-4) \times 10^{-5}$  G. Comparing these two estimates of the pressure we conclude that the magnetic and relativistic electron components of the jet fluid must be in approximate equipartition, and the thermal particles (though they may greatly outnumber the relativistic particles) cannot dominate the pressure. Furthermore, the radio emission cannot come from a region (for example, a thin surface layer or ribbon) involving some small fraction of the cross-section assumed on the basis of cylindrical symmetry.

The synchrotron lifetime for an electron emitting at 5 GHz in the equipartition field is  $\sim 5 \times 10^6$  yr which is of the order of 10 times the time for the ICM medium to flow past the jet and probably shorter than the flow time along the jet. This argues in favour of emission from the jet itself rather than from a sheath of shocked ICM and indicates the need to reaccelerate relativistic electrons *in situ*. The most surprising implication of this model, however, is the small fraction of the jet kinetic energy that is dissipated along its length. The precise relationship between the radio emissivity and the jet geometry obviously depends on very specific details of the model. In almost every hypothesis, however, the transverse extent of the radio contours would correspond at least to a scale  $h$ . The narrowness of the observed radio jet then implies that  $(h/R) \leq 0.05$ , so that  $\leq M_j^{-2} \approx 0.05$  of its kinetic energy can have gone into internal energy. Unless, for example the jet is a powerful millimetre source, it is impossible that a large fraction of the jet kinetic energy be radiated away, because the total observed jet luminosity is  $\leq 0.02$  of the radio power of the tail. These considerations in turn imply that the fraction of the ICM material impacting on the beam which can be entrained must be  $\leq M_j^{-2} (V_g/V_j)$ .

There is therefore observational support for the simple dynamical model of the jet outlines above. We have integrated equation (1) for a specific heat ratio of 5/3 (using  $\gamma = 4/3$  makes little difference). The resulting flow must be projected onto the sky. We have used  $\cos i = 0.8$  and varied the angle  $j$  between the jet axis and the line of sight to obtain a rough fit to the shape of the observed radio contours. This is shown in Fig. 2. We find that  $j \sim 60^\circ$ . If the jet angular width near the galaxy is  $\delta$ , then  $M_0 \sim 6(\delta/l'')^{-1/2}$ , and the existence of unresolved features  $\leq 0.5''$  in size suggests that  $M_0 \geq 8$ . However, if we take the width of a smooth trajectory enveloping the faintest contour, then we obtain  $M_0 \sim 4$ . The angle between the jet axis and the galaxy's velocity is  $\sim 80^\circ$ , and it is the closeness of this to  $90^\circ$  that is perhaps responsible for the unusually symmetric appearance of NGC1265.

Polarisation observations can in principle provide information on the density and hence  $V_j$ . The observations of Owen *et al.*<sup>8</sup> do not provide polarisation data but if the reported 30% polarisation<sup>7</sup> is due to the narrow jet, it implies highly ordered internal field structure, probably aligned along the jet by shearing motions. If the thermal plasma is uniformly distributed throughout the jet and not contained in dense clouds, then lack of depolarisation implies that  $n_i \leq 0.06$ . This is not a very significant limit. However, if the same level of polarisation in the narrow jet persisted down to lower frequencies it would set a more stringent limit on  $n_i$ , and thereby set a lower limit on  $V_j$ . If, for instance, the jet remained highly polarised down to 400 MHz,  $n_i$  would have to be so low that the required momentum flux could be supplied only if  $V_j$  exceeded  $\sim 10^6$  km s $^{-1}$  (a value that seems likely on other grounds).

The total luminosity of the tail is  $\sim 6 \times 10^{41}$  erg s $^{-1}$ . There is unlikely to be sufficient kinetic energy flux associated with the

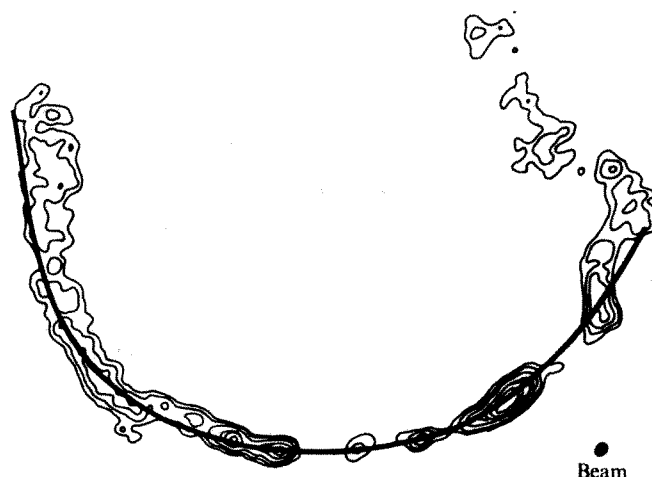


Fig. 2 VLA map of 3C83.1B at 4,886 MHz reproduced from Owen *et al.*<sup>8</sup>; only contours in the vicinity of the jet have been drawn. The shaded ellipse represents the half power contour of the clean beam, corresponding to a resolution of  $1.5'' \times 1.0''$ . Superimposed on this map is a trajectory obtained from integrating equation (1).

ICM to supply this. If this energy were all supplied by the jet then even assuming 100% efficiency we would infer, from our estimate of the momentum flux, a lower bound on  $V_j$  of 4,000  $(\delta/l'')^{-1} (n_c/3 \times 10^{-4} \text{ cm}^{-3})^{-1/2}$  km s $^{-1}$ . The jets appear (at least on one side) to break up after bending through  $\sim 75^\circ$ . The jet material may then share its momentum with the shocked ICM downstream (M.C. Begelman, in preparation), the energy thereby dissipated going into the production of a sheared magnetic field and into particle acceleration. The magnetic field strength within the tail region (assuming equipartition between the thermal gas, relativistic electron and magnetic energy, and pressure balance with the ambient ICM pressure) is  $\sim 10^{-5} (n_c/3 \times 10^{-4} \text{ cm}^{-3})^{1/2} (T_c/5 \times 10^7 \text{ K})^{1/2}$  G; lower by a factor  $\sim M_*$  than the field strength in the jet and slightly greater than the equipartition value inferred from observations. The tail has a width  $W \sim 40$  kpc and is highly polarised with a rotation measure  $\sim 30$  rad m $^{-2}$ . If the magnetic field is aligned parallel to the tail, as is expected if it is amplified by shear, then we can estimate the tail electron density to be  $n_t \sim 10^{-4} \text{ cm}^{-3}$ . For comparison, the mean electron density in the jet plasma is only  $\sim 10^{-6} (\delta/l'')^{-1} (V_j/10,000 \text{ km s}^{-1})^{-1} \text{ cm}^{-3}$  and so there must be some mixing with the ICM. In any case,  $n_t$  is smaller than  $n_c$ ; hence this model supports the idea that buoyancy is important in shaping the outermost parts of the tail<sup>16,20</sup>.

## General implications

Our basic starting point has been to assume that the tail of NGC1265 is energised by a 'twin-jet' emerging from the nucleus. This assumption is supported by the smooth arc-shaped curvature manifested in the high-resolution radio maps, the existence of an active radio nucleus in NGC1265, and by analogies with normal double sources. This interpretation requires that the jets be surprisingly stable and resistant to Kelvin-Helmholtz instabilities: the intergalactic wind flows across the beam with very little mixing or entrainment. The jets are able to maintain a Mach number  $\geq 4$  whilst being curved through an angle  $\sim 70^\circ$ . We would conjecture that the real flow pattern has no sharp velocity discontinuities: small-scale instabilities would certainly be inhibited if the shear layer actually had a thickness comparable with  $h$ .

We note also that the density profile across the jet is unlikely to follow the simple exponential law that we have assumed in our explicit model. Moreover, the flow pattern of the intergalactic medium through the galaxy is presumably much more complex than the uniform wind we have considered; even if there is not a large 'dead zone' of gas trapped in the galaxy's potential well, the flow pattern is likely to be modified by the entrainment of gas



resulting from stellar mass-loss in the galaxy. Our estimates of the energy flux and momentum flux along the jets are, fortunately, insensitive to these details. From the power requirements in the tail, we can guess that  $V_j \approx 10^4 \text{ km s}^{-1}$ . This corresponds to a flow time along the jet of  $2 \times 10^6 \text{ yr}$ , a power  $\sim 2 \times 10^{42} (n_c/3 \times 10^{-4} \text{ cm}^{-3})(\delta/1'')(V_j/10^4 \text{ km s}^{-1}) \text{ erg s}^{-1}$  and a mass discharge  $\sim 0.05 (n_c/3 \times 10^{-4} \text{ cm}^{-3})(\delta/1'')(V_j/10^4 \text{ km s}^{-1})^{-1} M_\odot \text{ yr}^{-1}$ . The energy and mass fluxes required of the nucleus are very small compared with those of the most powerful doubles.

The irregularities in the radio contours delineating the jet path imply either that its properties are unsteady or that there are instabilities along its path. The latter would not be unexpected—what seems astonishing is that the jet is stable enough to persist at all when exposed to a strong transverse force. Variations in the central energy supply—involving changes in  $V_j$  or in the internal properties of the ejecta—will also lead to nonuniformities along the track of the jet. If the jet were ever to switch off, the channel which it had made would persist for a time  $\sim 3h/V_j \sim 10^6 \text{ yr}$ . Variations on timescales less than this would lead merely to inhomogeneity but if the nuclear activity revived after a dormant phase longer than this, the jet would need to be re-excavated; a bow shock would advance out at a speed  $\sim M_j V_g$  as in the standard model for a double radio source component.

We have shown how the high resolution observations of NGC1265 may be interpreted in the context of a jet model and have constructed a simple equilibrium gas flow to describe the overall morphology. It remains to be shown that this flow is not subject to serious disruptive instabilities until the jet has bent through an angle  $\geq 70^\circ$ . The best hope for investigating

empirically the importance of these instabilities is perhaps with laboratory wind-tunnel experiments.

Finally, we note that, although other radio trails may not show such symmetric arc-shaped central structure when studied with  $\sim 1''$  resolution,<sup>21</sup> the same qualitative picture can apply. NGC1265 must be unusual in that not only do its velocity and beam axis both have substantial components transverse to the line of sight, but, furthermore, the beams happen to be directed almost perpendicular to the galaxy's motion through the IGM.

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## Sequence, structure and activity of phosphoglycerate kinase: a possible hinge-bending enzyme

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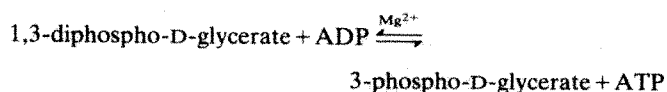
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*The fitting of sequenced peptides to a high-resolution X-ray map of phosphoglycerate kinase has yielded the complete sequence and structure of the horse muscle enzyme. Metal ADP and ATP substrates are bound to one of the two widely separated domains in an environment that seems unsuitable for phosphoglycerate binding. The most plausible binding site for the phosphoglycerate substrate is on the other domain about 10 Å from the ATP, which implies the possibility of a large scale hinge-bending of the domains to bring the two substrates together in a water-free environment for catalysis.*

PHOSPHOGLYCERATE KINASE (EC 2.7.2.3; PGK), catalyses the high-energy phosphoryl transfer reaction:



The enzyme is required for ATP generation in the glycolytic

pathways of aerobes and anaerobes, and for carbon fixation in plants. PGKs isolated from a wide variety of sources are monomeric with molecular weights around 45,000, with comparable amino acid compositions and similar catalytic properties. This has led to the proposal<sup>1</sup> that the enzyme has a highly conserved molecular and active site structure.

Preliminary X-ray studies of the horse muscle<sup>2,3</sup> and yeast enzymes<sup>4,5</sup> have shown that they are structurally homologous. The most remarkable feature of the PGK molecule is that its single polypeptide chain is organised into two widely separated domains of almost equal size. The results we report here suggest that this feature of the tertiary structure is probably associated with a large-scale 'hinge-bending' conformational change in the enzyme that is broadly similar to that previously reported for hexokinase<sup>22</sup>.

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## Experimental methods

Initially, a three-dimensional electron density map of horse PGK was obtained by conventional isomorphous replacement, using two derivatives, ethylmercuric phosphate and ethylmercuric chloride, each of which react with up to six of the enzyme's seven sulphhydryl groups. The X-ray intensities of the native enzyme and the two derivatives were collected on a five-counter, five-circle diffractometer<sup>7</sup> to 2.5 Å resolution. The data were corrected for Lorenz polarisation and radiation damage, and for absorption using the Huber-Kopfmann technique<sup>8</sup>. The phases of the 13,000 structure factors were estimated using the isomorphous and anomalous contributions of the heavy atoms. In parallel with the incorporation of the side-chain information from the sequenced peptides (see below), the protein structure was subjected to crystallographic refinement using the Hendrickson-Konnert constrained least squares program<sup>9</sup>, and the resultant calculated phases combined with the isomorphous phases using Hendrickson's method<sup>10</sup> to produce improved electron density maps. This procedure was carried out in three stages using: (1) main-chain atoms only (including  $\beta$ -carbons); (2) main chain + 200 available side chains; (3) main chain + 400 available side chains. As shown in Table 1 at each stage the conventional *R* factor for the refinement decreased, the figure-of-merit of the combined phases increased, and the new electron density maps improved markedly in interpretability.

The sequence analysis was carried out using both automated and manual Edman degradation of cyanogen bromide peptides and fragments derived from them by digestion with proteolytic enzymes. Treatment of horse muscle PGK with cyanogen bromide produced the 14 peptides consistent with the presence of 13 methionine residues in the molecule. The cyanogen bromide peptides were separated by gel filtration, ion-exchange chromatography, and where necessary, paper electrophoresis. The sequences of the individual peptides were obtained by a combination of automated Edman degradation using a Beckman 890C Sequenator, and the dansyl-Edman technique<sup>11</sup>. As with the enzymes from yeast, rabbit muscle<sup>12</sup> and human erythrocytes<sup>13</sup>, horse muscle PGK was found to have a blocked N-terminus. Thermolytic digestion of the N-terminal cyanogen bromide peptide revealed an *N*-substituted serine derivative, which was shown to be *N*-acetylserine by mass spectrometry.

Alignment of the sequenced cyanogen bromide fragments was carried out on the X-ray map. The six peptides containing more than 30 residues were fitted first. Their possible location on the molecule was determined using the following criteria: (1) each peptide began and ended at plausible methionine residues in the map; (2) any cysteine residues present in the sequence were located near one of the six known sites of mercurial binding; (3) Phe, Tyr and Trp residues in the sequence coincided with large side-chain densities; and (4) each of the 12 internal  $\beta$ -strands in the map corresponded to consecutive sequences of

four to six hydrophobic residues. The remaining eight peptides, which had 20 or fewer residues, often did not fulfil a sufficient number of criteria for an independent fit to be made with much confidence. Instead, when the sequence or lengths of all of them were available, they were fitted into the gaps between the longer peptides. Each fit was considered confirmed only when, in addition to all other criteria, it was found that internally positioned side-chains corresponded almost without exception to hydrophobic residues, and there was a good match of size and shape of the sequenced residues to their electron densities.

The 14 peptides were accommodated on the X-ray map in an end-to-end fashion, covering the complete polypeptide chain visible in the map. This fit is shown in Fig. 1, with the methionine residues marked to indicate the linkage of the sequenced peptides. The total of 416 residues in the polypeptide chain of horse muscle PGK corresponds to a molecular weight of 44,519, in good agreement with independent determinations<sup>1</sup>; and the total numbers of each type of amino acid residue are consistent with the overall amino acid composition<sup>14</sup>. Preliminary comparison of this sequence with peptide sequences from yeast PGK suggests a considerable degree of homology between the enzymes (L. A. Fothergill, personal communication).

## Molecular structure

Figure 2 shows a drawing in the conventional arrow and cylinder form of the tertiary structure of phosphoglycerate kinase. The division of the molecule into two discrete, separated globular domains can be seen clearly. It is now evident that the domains correspond to the N-terminal and C-terminal halves of the polypeptide chain, with the sole exception that the final 12 residues of the chain, forming helix 15, are packed in the N-terminal domain. There are therefore two links between the domains; the major one represented by residues 186–189 that form the junction between  $\beta$ -strand F and helix 7; and the minor one around residues 404–406 that connects helices 14 and 15. It is interesting to note that no element of regular secondary structure crosses the domain interface: strand F is clearly part of the N-domain, whereas helix 7 is associated with the C-domain; helices 15 and 14, respectively, are similarly disposed.

The N- and C-domains are almost precisely the same size and each is composed of a central  $\beta$ -sheet of six parallel strands surrounded by helices. In topological terms the order of the  $\beta$ -strands in the C-domain (CBADEF) is the same as that in the NAD-binding domain of the dehydrogenases<sup>15</sup>; whereas the order in the N-domain (CDBAEF) differs only in the location of strand D. There are, however, no significant similarities in the sequences of the two domains and their detailed structures are different. As in other glycolytic enzymes<sup>6</sup> there is a marked tendency for the secondary structural elements to be organised as repeating ( $\beta$ - $\alpha$ ) units<sup>16</sup>: in PGK the ( $\beta$ - $\alpha$ ) motif is repeated 12 times.

In total, the PGK molecule has 15 helices, 12 internal  $\beta$ -strands, and at least five surface  $\beta$ -strands. On the basis of the number and extent of the secondary structural elements shown in Figs 1 and 2, the PGK molecule has a minimum of 104 residues located in  $\beta$ -structures, and 174 in helices: these numbers represent 25% and 42% respectively of the residues in the molecule.

## Substrate binding

Horse PGK crystals grown in ammonium sulphate and equilibrated in potassium tartrate bind AMP and the complexes of ADP and ATP with  $Mg^{2+}$  or  $Mn^{2+}$ , at a single site on the C-terminal domain. The difference maps of MgATP at 3 Å resolution, and of MnATP at 2.5 Å resolution are sufficiently detailed for the conformation<sup>17</sup> of the bound nucleotide to be determined. The base is in the *anti* conformation with respect to the sugar, ( $\chi \sim 80^\circ$ ); the sugar pucker is C3' *endo*; and the conformation about the exocyclic C4'–C5' bond is *gauche* (–) (or *trans-gauche*). The triphosphate chain of ATP is in a somewhat coiled conformation and directed away from the adenine

**Table 1** Statistics of the X-ray analysis

Phase set	No. of atoms	<i>R</i> *	Figure of merit
Isomorphous	—	—	0.46
Combined set 1	1969 (main chain)	0.56	0.54
Combined set 2	2472 (main chain + 200 side-chains)	0.45	0.62
Combined set 3	3016 (main chain + 400 side-chains)	0.32	0.73
No. of intensities measured for native set to 2.5 Å			
		32,900	
No. of unique reflections to 2.5 Å		13,000	
Merging <i>R</i> † for native set		0.04	

$$R^* = \frac{\sum (F_{\text{obs}} - F_{\text{calc}})}{\sum F_{\text{obs}}} \quad R^\dagger = \frac{\sum (I_{\text{obs}} - I_{\text{mean}})}{\sum I_{\text{obs}}}$$

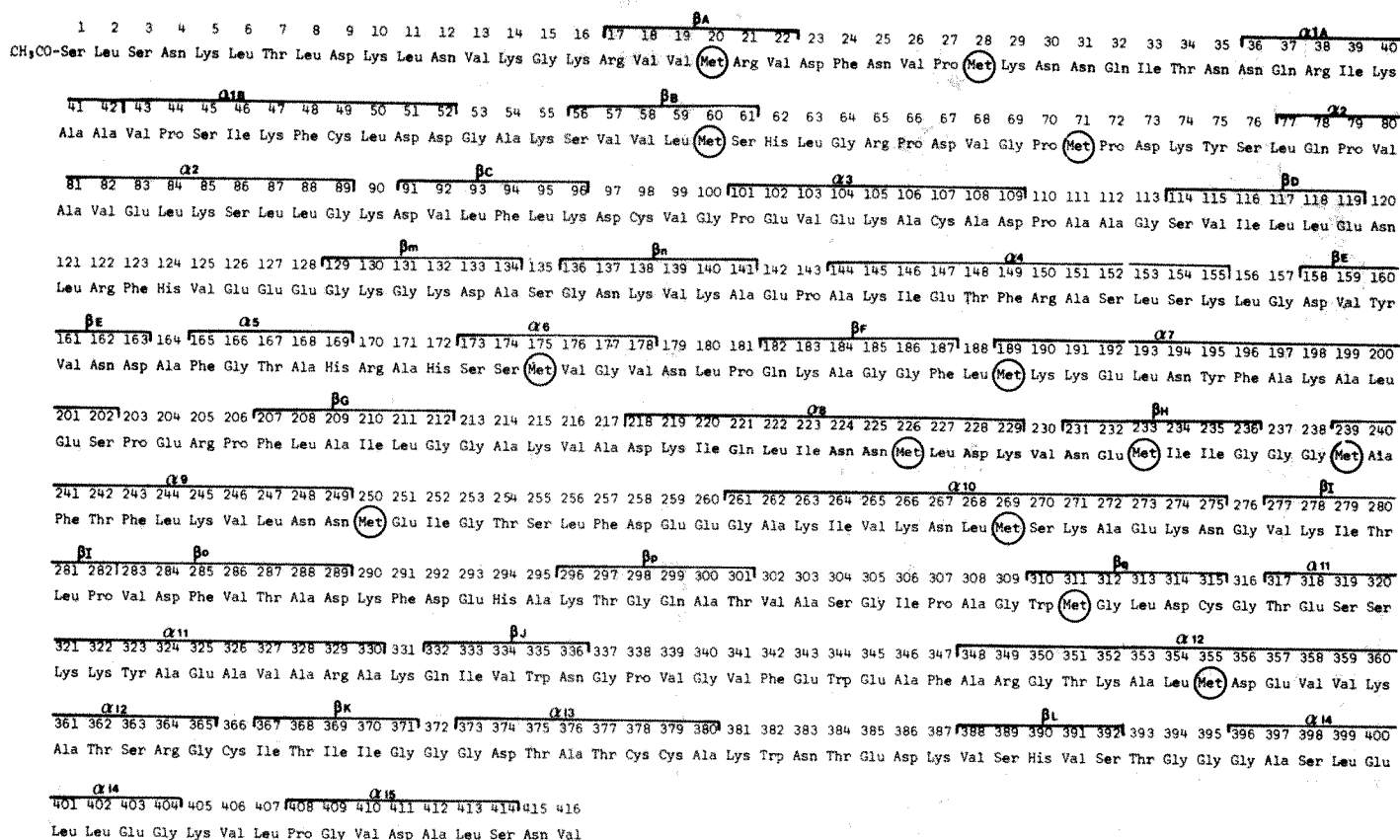


Fig. 1 Sequence of horse muscle phosphoglycerate kinase determined by ordering the 14 cyanogen bromide peptides on the X-ray map. The methionine residues that terminate each peptide are circled. The position and extent of the  $\alpha$ -helices and  $\beta$ -strands are indicated, using the same nomenclature as in Fig. 2.

group. This conformation can be seen in Fig. 4; it is quite different from the conformation observed in crystals of ATP<sup>18</sup>. Bound ADP has the same conformation as bound ATP.

The major interactions between the enzyme and the nucleotides are shown in Fig. 4. The adenine ring is almost completely buried in a deep, narrow slot. The amino group is innermost and probably makes a hydrogen bond with main-chain carbonyl of Leu 313. The adenine slot is lined by the main-chain segments that immediately follow  $\beta$ -strands G, H and J: Gly 212, Gly 213 and Ala 214; Gly 236, Gly 237 and Gly 238; and Val 339, Gly 340 and Val 341, giving the site a similar character to the aromatic specificity site of chymotrypsin<sup>19</sup>. The ribose is located in a shallow depression above the pyrrolidine ring of Pro 338. Ribose binding involves the side chain of Glu 343 which appears to move and stiffen to form hydrogen bonds with probably both the 2'- and 3'-hydroxyls of the sugar. The  $\alpha$ -phosphate group seems to make an ion-pair interaction with the charged  $\epsilon$ -amino group of Lys 219. The  $\beta$ - and  $\gamma$ -phosphates of ATP are located about 5 Å from the amino-terminus of helix 13, but do not interact with any side-chains. Hol *et al.*<sup>20</sup> have recently shown that the phosphate moieties of bound NAD, FAD and other phosphate-containing ligands, are similarly positioned in relation to helices in other enzymes, and suggest that there is a favourable ionic interaction between the negatively charged phosphates and the positive dipole at the N-termini of helices. It seems reasonable to conclude therefore that helix 13 in PGK acts as a phosphate-binding helix as suggested by Hol *et al.*<sup>20</sup>.

The binding of metal-ADP and -ATP complexes to PGK is accompanied by very similar small conformational changes in the vicinity of the binding site, and the only major difference between the interaction of the two nucleotides with the enzyme is in the location of the metal. Difference maps of the bound MgADP complex at 3 Å resolution in both sulphate and tartrate show a peak almost resolved from the main ADP density as shown in Fig. 3a. This peak coincides with the major peak in a

difference map at 6 Å resolution between MnADP and MgADP, and can therefore be reasonably ascribed to the metal ion. It is located about 4 Å from both the  $\alpha$ - and  $\beta$ -phosphates, and close to the carboxylate of Asp 374 as shown in Fig. 4. In contrast none of the difference maps of metal-ATP complexes shows a peak at this position (see Fig. 3b). Instead, difference maps of MnATP at 2.5 Å resolution and of MnATP-MgATP at 6 Å resolution show low peaks about 4 Å from the  $\gamma$ -phosphate. This evidence suggests that the metal may be associated with the  $\gamma$ -phosphate of ATP as shown in Fig. 4.

This X-ray picture of the nucleotide binding site on horse PGK is in excellent agreement with that produced for the yeast enzyme from the NMR studies of lanthanide ADP and ATP complexes<sup>21</sup>. The conformation of bound ATP revealed by both techniques is essentially similar, and the close cluster of three

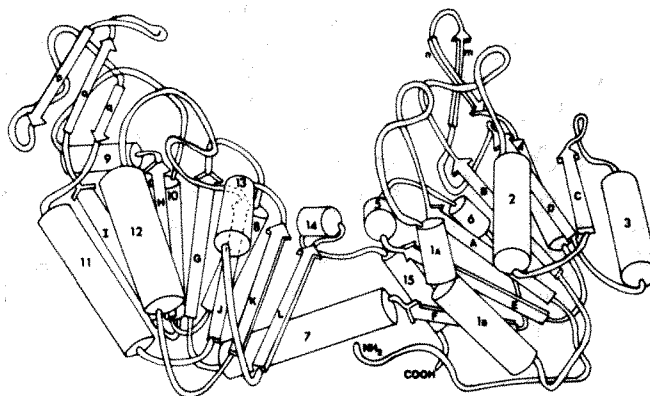
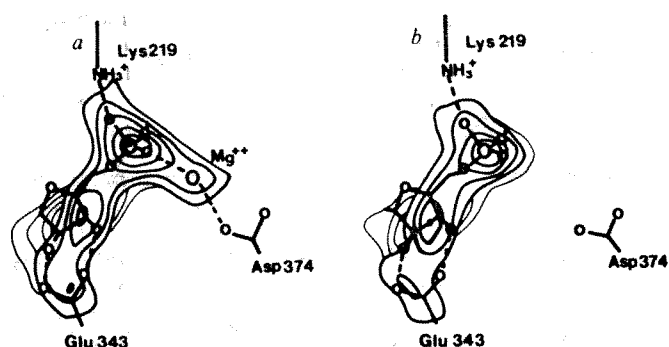


Fig. 2 A drawing of the structure of the native horse muscle phosphoglycerate kinase molecule.  $\alpha$ -Helices are defined by cylinders and the  $\beta$ -strands by arrows which also denote their direction.





**Fig. 3** Equivalent two sections through the difference maps of *a*, MnATP at 2.5 Å resolution and *b*, MgADP at 3.0 Å resolution, approximately in the plane of the ribose and  $\alpha$ -phosphate (see Fig. 4). The adenine is below the plane to the left and the remaining phosphates above the plane to the right. The peak corresponding to the metal in ADP can be clearly seen, as can its absence from this position in ATP. Interchange of  $Mg^{2+}$  for  $Mn^{2+}$  has no effect on the metal position, nor on enzyme activity<sup>12</sup>.

histidines located by NMR can be reasonably identified with the equally close cluster containing His 62, His 169 and His 172 shown in Fig. 4 by their similar position relative to the nucleotides.

In contrast to PGK crystals in sulphate, which do not interact with 3-phosphoglycerate even at high concentrations<sup>2,3</sup>, the same crystals in tartrate exhibit large changes to their diffraction patterns at quite low concentrations of this substrate. However, difference maps calculated at 6 Å resolution reveal that the intensity changes are almost entirely accounted for by conformational changes that occur throughout the enzyme and most probably correspond to a rotation of the two domains relative to one another. Unfortunately it is not possible amongst the numerous peaks corresponding to the conformational change to identify any single peak as representing the bound substrate. A difference map calculated at 6 Å resolution of the ATP-3-phosphoglycerate ternary complex is similar to the sum of the difference maps of the two equivalent binary complexes.

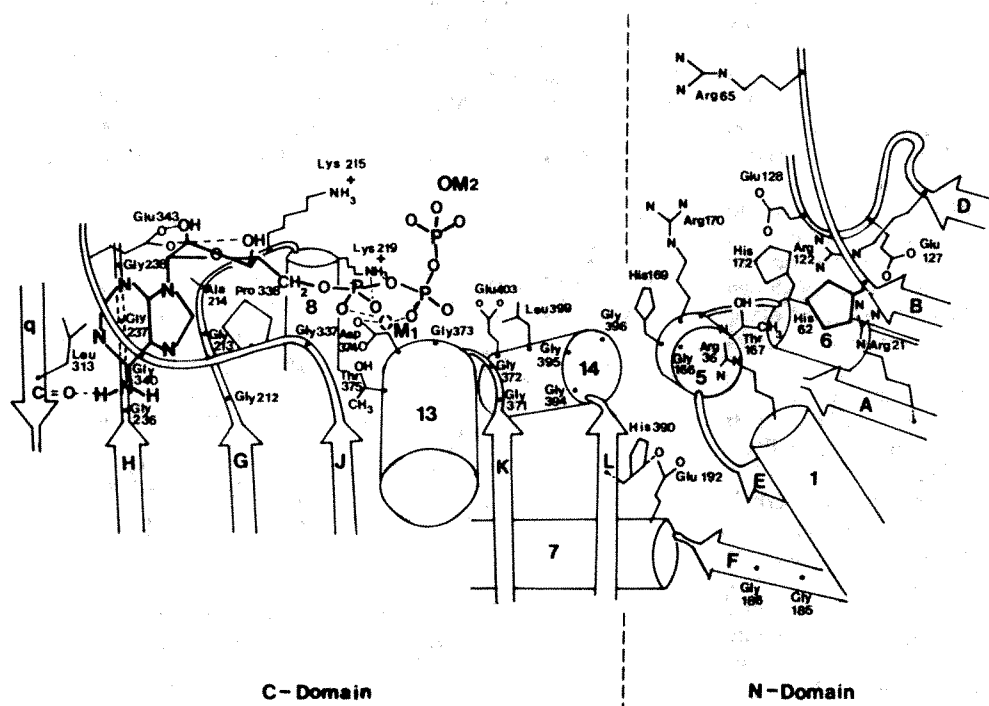
### Possible mode of action

It would be expected that location and definition of the binding site for one substrate in a two-substrate enzyme such as PGK

would lead to the identification, at least in general terms, of the binding site of the second substrate, and the catalytic site. This is not so on either count for PGK, as an examination of Fig. 4 shows. ATP is bound with its  $\gamma$ -phosphate, which is transferred by the enzyme, in a region of the molecule almost devoid of functional groups, and almost completely exposed to the solvent. The amino acids closest to the  $\gamma$ -phosphate include two triple glycine sequences, residues 371–373 and 394–396, and Leu 399. The nearest functional groups, Arg 38, His 169, Lys 215 and Glu 403 are about 10 Å distant. In this environment it is very difficult to discover any plausible binding site for 3-phosphoglycerate sufficiently close to the ATP for direct transfer of the phosphoryl group to occur; or any functional groups close enough to the  $\gamma$ -phosphate of the ATP to catalyse the transfer; or finally, how water could be excluded from the vicinity of the  $\gamma$ -phosphate during the catalytic reaction.

There seem to be three possible explanations of this apparent paradox: (1) the observed ATP does not represent the phosphorylating nucleotide; (2) the observed domain geometry of the enzyme is an artefact of crystallisation; (3) the phosphoglycerate substrates bind at a site distant from the nucleotide site. Although in relation to the first possibility the presence of more than one substrate binding site has been proposed to explain the nonlinear kinetics of homologous yeast<sup>23</sup>, and human erythrocyte<sup>24</sup> enzymes (which may have a different origin as discussed later), the independent X-ray studies of the horse and yeast enzymes<sup>5</sup>, and NMR studies on the yeast enzyme<sup>21</sup> all reveal the same single binding site. The second possibility seems highly unlikely because yeast PGK, the crystal packing of which is quite different to the horse enzyme, exhibits the same disposition of domains<sup>6</sup>. The direct test of the third possibility, by the X-ray examination of complexes of the enzyme with phosphoglycerate, was inconclusive because the induction of extensive conformational changes obscured the binding site.

In the present absence of experimental evidence, we have examined the molecular structure for plausible phosphoglycerate binding sites. We have used the following criteria: (1) as at physiological pH, 3-phosphoglycerate carries three negative charges, and the more strongly bound 1,3-diphosphoglycerate four negative charges, it seems reasonable to suppose the presence of a cluster of basic residues in their binding site; (2) because X-ray analysis of enzymes has shown that substrates are almost always located in surface depressions, a cavity capable of accommodating the relatively small phosphoglycerates was



expected; (3) because there seems to be no catalytic residues near the  $\gamma$ -phosphate of the ATP, functional residues that could contribute to the catalysis may be located near the phosphoglycerate site. Using these criteria we found only one possible candidate for the binding site. This is shown in Fig. 4.

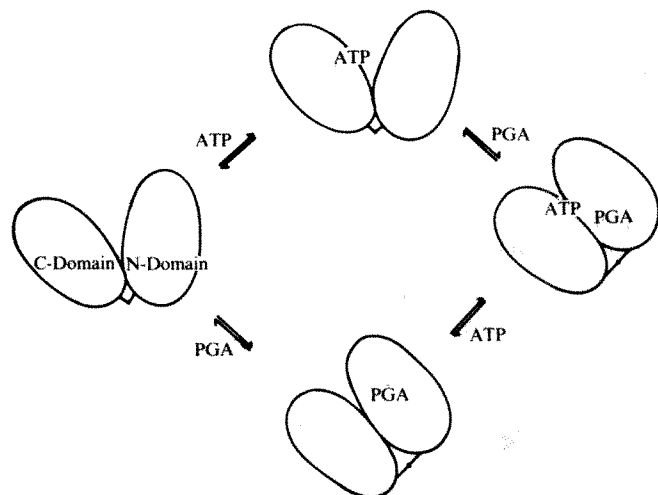


Fig. 5 Schematic drawing of the probable conformational changes in the substrate complexes of phosphoglycerate kinase suggested by the X-ray studies.

This site is located in the face of the N-terminal domain that opposes the ATP site on the C-terminal domain. The small cavity is located between the  $\beta$ -strands A and B and helix 6. Located within it and around it are five arginines, residues 21, 38, 65, 122 and 170; three histidines, residues 62, 169 and 172; two glutamates, residues 127 and 128; and aspartate, 23. Of these Arg 21 and Glu 127 are almost buried inside the cavity. This small region of the molecule contains half the arginine and histidine residues in the whole molecule. Note that in the NMR 'map' of the active site of yeast PGK<sup>21</sup>, the phosphoglycerate substrate is located further away from the ATP than the cluster of three histidines, which if identified with His 62, 169 and 172 of the horse enzyme, would place it in or near the cavity. If we assume that the observed structural homology between the yeast and horse enzymes extends to residues involved in substrate binding, the observation that at least one arginine takes part in the binding of 3-phosphoglycerate in the yeast enzyme<sup>26</sup> is also consistent with phosphoglycerate binding in the cavity.

If our tentative identification of the cavity with the phosphoglycerate site is correct, there is another problem to consider: as located on the native enzyme there must be a gap of about 10 Å between the  $\gamma$ -phosphate of the ATP and the carboxyl group of 3-phosphoglycerate that we presume must be closed when the phosphoryl group is catalytically transferred. Examination of the structure of the PGK molecule suggests that although each domain has a highly organised and apparently stable globular structure, the domain interface is small and not crossed by any of the secondary structures, suggesting that the enzyme is capable of a 'hinge-bending' conformational change. Consideration of Figs 2 and 4 shows that a rotation of 10–20° about a hinge located in or near the connection between  $\beta$ -strand F and helix 7 would be sufficient to bring the two domains, together with the ATP in its observed binding site, and the 3-phosphoglycerate in its proposed binding site, into contact, and at the same time to expel the solvent that lies between them. Residues 185 and 186 represent a Gly-Gly sequence at the C-terminus of  $\beta$ -strand F which could give the required degree of freedom at the appropriate position.

The 'hinge-bending' conformational change to PGK outlined above is illustrated in Fig. 5. We show the conformational change as occurring with the formation of phosphoglycerate

binary complexes, rather than with the formation of nucleotide binary complexes or ternary complexes, because our X-ray studies show that in the crystals in tartrate only small, local, conformational changes occur when ADP or ATP bind, whereas changes involving the whole molecule occur in the presence of 3-phosphoglycerate which are not increased in the ternary complex obtained by adding ATP. NMR studies of binary and ternary complexes of the yeast enzyme also show the same pattern of conformational change<sup>21</sup>, and studies on the rabbit muscle enzyme show that the reactivities of the seven sulphhydryl groups are affected to a greater extent by 3-phosphoglycerate than by ADP or ATP<sup>12</sup>. One consequence of the conformational change is the possibility that it could mediate in allowing the enzymatic activity to be modulated by substrates and non-substrates, giving rise to the nonlinear kinetics, and activation and inhibition phenomena that have been observed with the human<sup>23</sup> and yeast enzymes<sup>24,25</sup>, and which are otherwise difficult to reconcile with a monomeric enzyme. Although the 'hinge-bending' conformational change shown in Fig. 5 must be regarded as tentative until more information on the nature of binary and ternary enzyme-substrate complexes is obtained, it is consistent with most of the X-ray, NMR and chemical data discussed previously, some of which is difficult to understand in terms of a simple, monomeric enzyme.

Since preparing this paper we have become aware that Pickover *et al.*<sup>27</sup> have obtained direct evidence of a large conformational change in PGK from low angle X-ray studies of solutions of the yeast enzyme. They have found that the radius of gyration of the enzyme decreases by 1.09 Å on binding both ATP and 3-phosphoglycerate to form the ternary complex, but that binary complexes of these two substrates show much smaller decreases. Using the coordinates of the horse muscle enzyme they have interpreted the change in the radius of gyration in terms of a 'hinge-bending' conformational change of about 9–12° about a hinge located in the domain interface. Although the solution and crystal X-ray studies differ on whether the conformational change occurs on the formation of binary or ternary enzyme-substrate complexes, the proposed conformational changes are very similar: the illustration that Pickover *et al.*<sup>27</sup> provide of the enzyme after rotation of 12° about residue 187 (see Fig. 4) is extremely close to that which we have discussed above as being required to bring the observed ATP site and the proposed phosphoglycerate site together.

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# Cloned fragments of the plasmid ColV,I-K94 specifying virulence and serum resistance

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*A cloned BamHI-generated fragment of ColV,I-K94 increased the virulence of Escherichia coli, causing an approximately 100-fold reduction in LD<sub>50</sub> for chicks. A genetic determinant for resistance to the bactericidal effects of serum was mapped to a 5,300 base-pair sequence within the fragment. Neither colicin V nor immunity to colicin V affected the pathogenicity of E. coli for chicks.*

STRAINS of *Escherichia coli* are an important cause of both diarrhoea and generalised infections in man and livestock<sup>1</sup>. Enteropathogenic strains causing diarrhoea are usually confined to the alimentary tract and often harbour conjugative plasmids specifying enterotoxins<sup>2</sup> and K88 (or K89) antigen<sup>3</sup> which enhance their pathogenicity. Smith<sup>4,5</sup> found that plasmids could also enhance the virulence of invasive strains responsible for generalised infections. Most of the *E. coli* strains responsible for independent cases of generalised infections in calves, lambs or chicks produced colicin V, a plasmid-determined antibacterial protein. Elimination of the ColV plasmids from human, bovine, ovine and avian strains of several different serotypes invariably reduced their pathogenicity for experimental animals. Re-introduction of ColV plasmids into the strains by conjugation restored their pathogenicity to its original level. These results demonstrated that ColV plasmids increased the invasiveness of *E. coli*, but as Smith pointed out<sup>4</sup>, did not indicate whether colicin V itself or some other products specified by the ColV plasmids were responsible. The antibiotic effects of colicin V, like those of other colicins, are confined largely to enterobacteria; colicin V is not toxic for animals<sup>6,7</sup>. However, the ecological significance of colicins is obscure (see ref. 8) and it remained possible that colicin V might increase the virulence of *E. coli*.

## Isolation and characterisation of ColV derivatives

The genetic determinants for virulence and for increased survival in serum, which are probably identical as discussed below, were mapped by isolating deletion mutants and point mutants of ColV, I-K94 and by cloning fragments of the plasmid into the plasmid vector pBR322 (ref. 9) by *in vitro* recombination procedures. The ColV derivatives were isolated in *E. coli* K-2 strains and were then transferred into a recently isolated *E. coli* strain which was more suitable for testing the effects of plasmids on the pathogenicity of *E. coli* for experimental animals.

ColV, I-K94 is a conjugative (F-like) plasmid<sup>10-12</sup> specifying colicin I as well as colicin V<sup>11</sup>. It was originally found in *E. coli* K94, a strain isolated from the stools of a patient suffering from a *Salmonella paratyphi* B infection (ref. 13 and P. Fredericq, personal communication). The positions of the genes specifying colicins and colicin immunity in ColV, I-K94 (pKH38) and in its derivative pKH46-1::γδ have been determined (D.L.D., M.M.B. and K.G.H., in preparation). The genetic and physical maps of ColV, I-K94 and the derivatives used here are shown in Fig. 1.

Most of the plasmids used in the pathogenicity tests were derived from pKH46-1::γδ, a *tra*<sup>-</sup> deletion mutant of ColV, I-

K94 (pKH38) specifying colicins V and I. pKH46-1::γδ has an insert of the 5.7-kilobase γδ insertion sequence<sup>14,15</sup> and was isolated after mobilisation of pKH46 by the F plasmid. The presence of the γδ sequence in pKH46-1::γδ and its derivatives increased the frequency with which these plasmids could be transferred by F mobilisation<sup>15</sup> into the strain used for testing pathogenicity and serum resistance. This was an essential feature of these plasmids as the strain used for these tests (KH933, serotype 078:K80) was a poor recipient in matings with either derepressed F-like or I-like plasmids and could not be transformed (<1 transformant per 100 μg of supercoiled pKH46-1::γδ DNA using the procedure described by Cohen *et al.*<sup>16</sup>).

Spontaneous deletion mutants of pKH46-1::γδ which specified no colicin V or only reduced titres occurred at a frequency of approximately 0.1%. The deletions in the two mutants used here had one end in common, namely the end of one of the 2.1-kilobase inverted repeats in pKH46-1::γδ (Fig. 1). The positions of the deletions were determined by electrophoresis of fragments generated by restriction endonucleases, as described in Fig. 1 legend, and from heteroduplexes formed between the deletion mutants and derivatives of pKH46-1::γδ which had insertions of the transposon Tn5 (ref. 17) which specifies kanamycin resistance. The positions of Tn5 in these plasmids are shown in Fig. 1. Plasmid

**Table 1** Colicin synthesis and immunity specified by derivatives of ColV, I-K94

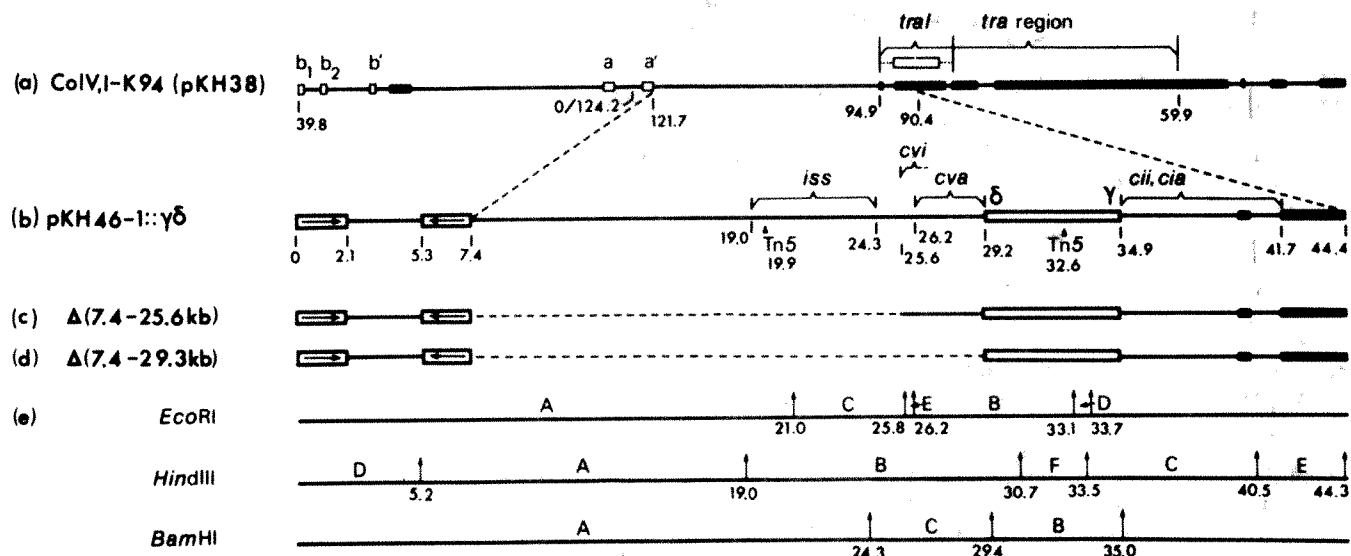
Strain	Plasmid	Colicin titres		Colicin immunity	
		V	I	V	I
KH961	pKH46-1::γδ	64	512	+	+
KH974	pKH46-1::γδ Δ (7.4-25.6 kilobases)	<1*	512	+	+
KH982	pKH46-1::γδ Δ (7.4-29.3 kilobases)	—	256	—	+
KH962	pKH46-1::γδ <i>cia</i> -1	32	1	+	+
KH1020	pKH46-1::γδ 19.9kb::Tn5	64	256	+	+
KH1022	pKH46-1::γδ 19.9kb::Tn5 <i>cua</i> -1	<1	256	+	+
KH1069	pBH11	32	—	+	—
KH1070	pBH14	—	—	—	—
KH1071	pBR322: <i>Bam</i> HI-A	—	4096	—	+
KH1072	pBR322: <i>Bam</i> HI-C	<1*	—	+	—
KH1073	pBR322: <i>Hind</i> III-B	64	—	+	—
KH1074	pBR322: <i>Eco</i> RI-A	—	2048	—	+
KH932	ColV-B188	<1*	—	+	—
KH933	None	—	—	—	—

The host strain for all plasmids was KH933 (serotype 078:K80). For colicin titrations, broth cultures grown to  $A_{600} = 0.8$  were disrupted by sonication for 35 s (ref. 24). Serial twofold dilutions of the sonicates were made and 0.02-ml volumes of each dilution were dropped onto the surface of 25-ml nutrient agar plates (Oxoid blood agar base no. 2) containing streptomycin (200 μg ml<sup>-1</sup>) which had been overlaid with 3 ml of nutrient agar containing colicin-sensitive bacteria at a concentration of  $2.5 \times 10^8$  cells ml<sup>-1</sup>. Colicin I was assayed on plates overlaid with P1235 (ref. 37), a colicin V-tolerant mutant. Colicin V was assayed on plates overlaid with strain KH960, a colicin I-tolerant mutant of AB1157. Colicin titres are expressed as the highest dilution which inhibited growth of indicator bacteria. Colicin immunity was determined by streaking a loopful of broth culture (in exponential phase at  $2 \times 10^8$  cells ml<sup>-1</sup>) across a streak of colicin-producing bacteria (KH982 or KH1069) which had been grown for 24 h on nutrient agar, chloroformed and then overlaid with 3 ml of nutrient agar. Growth of colicin-immune bacteria (labelled +) was not inhibited over the streak. — Indicates absence of genetic determinant. Immunity to colicin I was tested using *E. coli* K-12 (W3110) as a host strain since KH933 is resistant to colicin I.

\* These strains produced no detectable colicin V in broth cultures, but 48-h colonies on nutrient agar plates formed small inhibition zones when chloroformed and overlaid with strain KH960.

† Growth was partially inhibited by colicin V.





**Fig. 1** Physical and genetic maps of ColV, I-K94 and its derivatives. *a*, ColV, I-K94 (pKH38). The positions of inverted repeat sequences (shown as open boxes) and of sequences homologous to the F plasmid in pKH38/F heteroduplexes (shown as thicker lines) were determined by electron microscopy of plasmid DNA (D.L.D., M.M.B. & K.G.H., in preparation). Coordinates in pKH38 (total length 124.2 kilobases) were defined, as described by Sharp *et al.*<sup>32</sup>, with reference to the 0.8-kilobase insertion/deletion loop at 87.1 F in pKH38/F heteroduplexes. ColV, I-K94 (pKH38) differs from the ColV, I-K94 molecules examined by Sharp *et al.*<sup>32</sup> in the number and arrangement of 1.3-kilobase inverted repeat sequences and in not forming an insertion/deletion loop at 58.0 F in pKH38/F heteroduplexes. The *tra* region comprises the sequence which is homologous (as determined by heteroduplex analysis in the conditions described by Sharp *et al.*<sup>32</sup>) to the F plasmid sequence 62.0 F–93.2 F which is essential for conjugation<sup>33,34</sup>. Part of the ColV, I-K94 sequence which is homologous to the *traI* gene of the F plasmid occurs in pKH46-1:: $\gamma\delta$  as indicated in *b*. The *traI* gene of F maps within the homologous sequence indicated by dotted lines; the box represents the length of DNA sequence required to code for the protein specified by *traI* which has a molecular weight of 174,000 (ref. 34). *a,a'* Is an inverted repeat sequence of  $1.3 \pm 0.09$  kilobases; *b<sub>1</sub>,b<sub>2</sub>,b'* are three homologous sequences of  $0.8 \pm 0.08$  kilobases, two of which have the same orientation. *b*, pKH46-1:: $\gamma\delta$  is a deletion mutant of pKH38. The genes specifying colicin V (*cva*), immunity to colicin V (*cvi*), colicin I (*cii*), immunity to colicin I (*cii*) and increased survival in serum (*iss*) map within the regions indicated. The *cvi* gene maps to the left of *cva* as drawn, but the maximum limit of *cvi* to the right has not been determined. The positions of genes specifying colicins were determined from the analysis of cloned fragments and the relationship of pKH46-1:: $\gamma\delta$  to pKH38 was determined by heteroduplex analysis (D.L.D., M.M.B. & K.G.H., in preparation). The pKH46-1:: $\gamma\delta$  2.7–5.3-kilobase sequence is homologous to the sequence between the 1.3-kilobase inverted repeat sequence (*a,a'*) in pKH38, but in pKH46-1:: $\gamma\delta$  it is flanked by a  $2.1 \pm 0.1$ -kilobase inverted repeat sequence. The remaining 37 kilobases of pKH46-1:: $\gamma\delta$  (with the exception of the  $\gamma\delta$  insert) are homologous to the pKH38 90.4–121.7-kilobase sequence. pKH46-1:: $\gamma\delta$  coordinates are assigned from the end of one of the 2.1-kilobase inverted repeats. Copies of the Tn5 transposon were added to pKH46-1:: $\gamma\delta$  by mobilisation of the plasmid with *F'*<sub>lac</sub><sup>+</sup> (ref. 21) from a donor lysogenic for the bacteriophage  $\lambda$ b<sub>513</sub>b<sub>519</sub>x<sub>156</sub>Cl<sub>857</sub>S<sub>7</sub>rex::Tn5 (ref. 17) to a *recA*<sup>+</sup> recipient in matings at 30 °C. Two recipients selected for expression of kanamycin resistance at 42 °C harboured plasmids with Tn5 insertions at the positions indicated. *c* and *d* are deletion mutants, pKH46-1:: $\gamma\delta$   $\Delta$  (7.4–25.6 kilobases) and pKH46-1:: $\gamma\delta$   $\Delta$  (7.4–29.3 kilobases). Restriction endonuclease sites within pKH46-1:: $\gamma\delta$  and its derivatives (*e*) were determined by electrophoresis of fragments generated by complete or partial digestion with restriction endonucleases (Boehringer) used either alone or in combination. Restriction endonuclease reactions were carried out for 15 min at 37 °C in 50- $\mu$ l volumes containing approximately 0.5  $\mu$ g DNA in the following buffers: 100 mM Tris-HCl (pH 7.5), 50 mM NaCl, 10 mM MgCl<sub>2</sub> (for *Eco*RI); 10 mM Tris-HCl (pH 7.6), 50 mM NaCl, 10 mM MgCl<sub>2</sub>, 14 mM dithiothreitol (for *Hind*III); 6 mM Tris-HCl (pH 7.5), 20 mM KCl, 6 mM MgCl<sub>2</sub>, 6 mM dithiothreitol (for *Bam*HI). Fragments were analysed by electrophoresis through horizontal slab gels containing 0.8% (w/v) agarose and ethidium bromide (0.5  $\mu$ g ml<sup>-1</sup>) in Tris-borate buffer<sup>35</sup> at 60 V for 18 h at room temperature. Small fragments were analysed by polyacrylamide gel electrophoresis as described by Bolivar *et al.*<sup>9</sup>. Fragments of  $\lambda$ Cl<sub>857</sub>S<sub>7</sub> DNA generated by *Eco*RI and *Hind*III were included in gels as molecular weight standards<sup>36</sup>. Fragments of pKH46-1:: $\gamma\delta$  were ligated to pBR322 (ref. 9) in a reaction mixture containing 0.066 mM ATP, 66 mM Tris-HCl (pH 7.6), 6.6 mM MgCl<sub>2</sub>, 10 mM dithiothreitol, 1 unit T4 DNA ligase (Bethesda Research Laboratories) and 1  $\mu$ g of restriction endonuclease-cleaved plasmid DNA in a total volume of 50  $\mu$ l. After incubation for 4 h at 20 °C, recombinant plasmids were selected from the mixture by transformation<sup>16</sup> and selection on nutrient agar plates containing ampicillin at a concentration of 120  $\mu$ g ml<sup>-1</sup>. Experiments involving recombinant plasmids were carried out in Class I conditions as defined by the Williams report (Cmnd 6600).

pKH46-1:: $\gamma\delta$   $\Delta$  (7.4–25.6 kilobases) specified greatly reduced titres of colicin V and immunity to colicin V (Table 1) and pKH46-1:: $\gamma\delta$   $\Delta$  (7.4–29.3 kilobases) specified no colicin V or immunity to colicin V. A point mutant of pKH46-1:: $\gamma\delta$  19.9kb::Tn5 (pKH46-1:: $\gamma\delta$  containing the Tn5 transposon at the 19.9-kilobase position) which did not specify the synthesis of colicin V, was obtained by treating plasmid DNA at 37 °C for 24 h with 0.4 M hydroxylamine in the presence of 1 mM EDTA as described by Tessman<sup>18</sup>. A kanamycin-resistant transformant which did not specify colicin V synthesis (*Cva*<sup>-</sup>) was selected. The fragments of pKH46-1:: $\gamma\delta$  19.9kb::Tn5 *cva*-1 generated by *Eco*RI and by *Hind*III were identical to those of pKH46-1:: $\gamma\delta$  19.9kb::Tn5.

Fragments of pKH46-1:: $\gamma\delta$  obtained by digestion with restriction endonucleases *Eco*RI, *Hind*III or *Bam*HI were cloned in the plasmid vector pBR322 (ref. 9) as described in Fig. 1 legend. Recombinant plasmids containing the following fragments were isolated: *Eco*RI-A, *Bam*HI-A, *Bam*HI-C and *Hind*III-B. In addition, part of the *Hind*III-B fragment, comprising the 22.6–30.7-kilobase sequence of pKH46-1:: $\gamma\delta$ , was cloned in pBR322 to form pBH11. pBH11 was obtained as a transformant from a *Hind*III-digested preparation of pKH46-1:: $\gamma\delta$  which was incubated with T4 DNA ligase in the presence of *Hind*III-digested pBR322. However, pBH11 was found to

have only one *Hind*III site although it specified both colicin V synthesis and ampicillin resistance. Analyses of restriction endonuclease digests of pBH11 and of the heteroduplexes pBH11/pBR322 and pBH11/pKH46-1:: $\gamma\delta$  19.9kb::Tn5 established that the entire pBR322 sequence, but only part of the *Hind*III-B fragment, was present in pBH11. The orientation of the fragment in pBH11 was the same as in the plasmid in which the entire *Hind*III-B fragment had been cloned, that is, the sequence derived from the  $\gamma\delta$  insert (29.2–30.7 kilobases) was adjacent to the closely linked *Hind*III and *Eco*RI sites of pBR322. pBH14 was derived from pBH11 by ligating a 7.5-kilobase *Eco*RI-generated fragment of pBH11; it comprises the 22.6–25.8 kilobase sequence of pKH46-1:: $\gamma\delta$  and the pBR322 plasmid sequence with the exception of the 31-base pair sequence between the *Eco*RI and *Hind*III sites of pBR322.

### Transfer of cloned fragments and ColV mutants to an invasive strain by mobilisation

To determine their effects on the pathogenicity of *E. coli* for chicks, ColV derivatives were transferred by conjugation into strain KH933, a Col<sup>-</sup> derivative of *E. coli* B188. *E. coli* B188 was isolated from a calf suffering from a generalised infection and has the serotype 078:K80 (ref. 4), the most common

serotype among *E. coli* strains responsible for generalised infections in livestock<sup>1,4</sup>. The ColV plasmid originally present in *E. coli* B188 was cured by treatment of the strain with acridine orange<sup>19</sup> and its absence from the Col<sup>-</sup> derivative, KH933, was confirmed by dye-buoyant density centrifugation<sup>20</sup>. KH933 was chosen as the host strain for ColV derivatives so that the results of the pathogenicity tests could be compared with published data on the effects of other ColV plasmids<sup>4,5</sup>. Furthermore, the effects of plasmids on a strain known to be a potential pathogen would be more significant than their effects on the pathogenicity of a strain such as *E. coli* K-12 which kills experimental animals only when given in huge doses.

Plasmid pKH46-1:: $\gamma\delta$  and its derivatives which also comprised a copy of the  $\gamma\delta$  insertion sequence were transferred from auxotrophic *E. coli* K-12 donors using F<sub>10114</sub>lac<sup>+</sup> (ref. 21) as a mobilising plasmid in 8-h matings in broth at 30 °C. Recipients were selected on glucose-minimal salts agar<sup>22</sup> containing kanamycin at a concentration of 25  $\mu\text{g ml}^{-1}$  or colicin V (produced by a lawn of ColV<sup>+</sup> bacteria which was chloroformed after 18 h of growth, overlaid with 10 ml of agar and incubated for 4 h at 37 °C before being used for selection). F<sup>-</sup>Col<sup>+</sup> derivatives of the recipients were isolated from broth cultures which had been grown at 42 °C. Plasmid pKH46-1:: $\gamma\delta$   $\Delta$  (7.4–29.3 kilobases) was isolated as a spontaneous mutant from a KH933 strain harbouring pKH46-1:: $\gamma\delta$ .

Derivatives of pBR322 were transferred to KH933 from *E. coli* K-12 donors which also harboured the I-like conjugative plasmid R144drd3 (ref. 23) and the non-conjugative plasmid ColK-K235 (ref. 24). ColK-K235 provided the *trans*-acting mobility determinant<sup>25</sup> which is essential for efficient mobilisation of pBR322 derivatives by R144drd3. Matings were interrupted after 5 min. Approximately 25% of recipients selected on plates containing ampicillin at a concentration of 120  $\mu\text{g ml}^{-1}$  were ColK<sup>+</sup> and R<sup>+</sup>. Colicin titres specified by ColV derivatives in the host strain KH933 are listed in Table 1.

Table 2 Effect of ColV derivatives on the pathogenicity of *E. coli*

Strain	Plasmid	LD <sub>50</sub> ( $\pm$ s.e.m.) $\times 10^{-5}$
KH932	ColV-B188	2.4( $\pm$ 2.4)
KH961	pKH46-1:: $\gamma\delta$	2.1( $\pm$ 1.1)
KH962	pKH46-1:: $\gamma\delta$ <i>cia</i> -1	2.0( $\pm$ 2.1)
KH1020	pKH46-1:: $\gamma\delta$ 19.9kb::Tn5	1.0( $\pm$ 1.7)
KH1071	pBR322: <i>Bam</i> HI-A	1.8( $\pm$ 1.8)
KH1022	pKH46-1:: $\gamma\delta$ 19.9kb::Tn5 <i>cva</i> -1	6.2( $\pm$ 3.2)
KH974	pKH46-1:: $\gamma\delta$ $\Delta$ (7.4–25.6 kilobases)	220( $\pm$ 170)
KH982	pKH46-1:: $\gamma\delta$ $\Delta$ (7.4–29.3 kilobases)	490( $\pm$ 240)
KH1069	pBH11	380( $\pm$ 200)
KH1070	pBH14	180( $\pm$ 140)
KH1075	None	320( $\pm$ 120)
KH933	None	190( $\pm$ 180)

Pathogenicity was tested by injecting appropriate dilutions of broth cultures intramuscularly into 1-d-old chicks. For each strain, 14 chicks were injected at each of three or four concentrations of bacteria, ranging in 10-fold increments from 10<sup>5</sup> to 10<sup>8</sup> viable cells. Survival was recorded after 7 days. LD<sub>50</sub> values  $\pm$  s.e.m. were calculated by probit analysis as described by Finney<sup>26</sup>. KH1075 is a derivative of KH1069 which had spontaneously lost pBH11.

## Pathogenicity of strains for chicks

Pathogenicity tests of strains for 1-d-old chicks (Table 2) showed that neither of the deletion mutants of pKH46-1:: $\gamma\delta$  nor pBH11, which specified high levels of colicin V, affected the pathogenicity of KH933. However, pBR322 linked to the *Bam*HI-generated fragment A (subsequently referred to as pBR322:*Bam*HI-A), which specified neither colicin V nor immunity to colicin V, increased the pathogenicity of KH933 as much as did pKH46-1:: $\gamma\delta$ , pKH46-1:: $\gamma\delta$  19.9kb::Tn5, pKH46-1:: $\gamma\delta$  *cia*-1 or ColV-B188. All these plasmids produced an approximately 100-fold decrease in the LD<sub>50</sub> of strain KH933 for 1-d-old chicks. The mutation in pKH46-1:: $\gamma\delta$  19.9kb::Tn5 *cva*-1 which affected colicin V synthesis did not significantly affect the ability of the plasmid to increase the

virulence of *E. coli*. As pBR322:*Bam*HI-A increased pathogenicity but pKH46-1:: $\gamma\delta$   $\Delta$  (7.4–25.6 kilobases) did not, the virulence determinant can be mapped to the 7.4–24.3-kilobase sequence in pKH46-1:: $\gamma\delta$  and to the 104.8–121.7-kilobase sequence in ColV, I-K94 (pKH38). The slopes of the dose-response curves for all the strains tested were not significantly different; the ratios of the LD<sub>50</sub> values can therefore be used to compare the pathogenicity of strains.

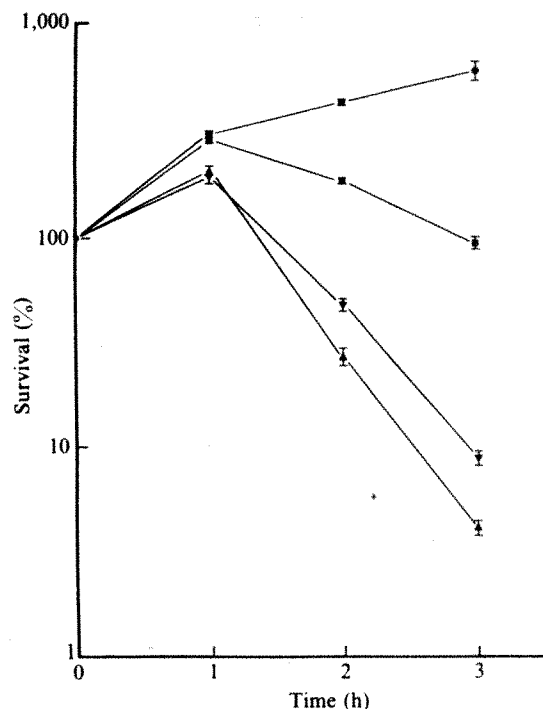
## Survival of strains in serum

Smith<sup>4</sup> found that ColV<sup>+</sup> strains survived better in a variety of animal sera than did their Col<sup>-</sup> counterparts. Strains harbouring ColV derivatives were therefore examined to determine the position of the genetic determinant for increased survival in serum (*iss*). We used fresh rabbit serum, but Smith<sup>4</sup> has previously shown that ColV plasmids also enhance survival in chicken serum. In addition to the plasmids used in the pathogenicity tests, the effects of the cloned fragments, *Hind*III-B, *Eco*RI-A and *Bam*HI-C, on the survival of KH933 in rabbit serum were examined. Of the 14 plasmids tested, the following did not affect the survival of KH933 in fresh serum: pKH46-1:: $\gamma\delta$   $\Delta$  (7.4–25.6 kilobases), pKH46-1:: $\gamma\delta$   $\Delta$  (7.4–29.3 kilobases), pBR322, pBH14, pBR322:*Eco*RI-A, pBR322:*Bam*HI-C. pBH11 caused a small but significant increase in survival (Fig. 2). The following plasmids greatly increased the survival of KH933 in serum: pKH46-1:: $\gamma\delta$ , pKH46-1:: $\gamma\delta$  19.9kb::Tn5, pKH46-1:: $\gamma\delta$  *cia*-1, pKH46-1:: $\gamma\delta$  19.9kb::Tn5 *cva*-1, pBR322:*Hind*III-B, pBR322:*Bam*HI-A and ColV-B188. All these plasmids produced a similar increase in serum resistance, with the exception of pBR322:*Bam*HI-A, which was more effective than any of the others (Fig. 2). pBR322 is a multi-copy plasmid<sup>9</sup>; the high level of serum resistance specified by pBR322:*Bam*HI-A may therefore result from multiple copies of the *iss*<sup>+</sup> determinant. The titres of colicin I specified by pBR322:*Bam*HI-A were eightfold greater than those specified by pKH46-1:: $\gamma\delta$  (Table 1). A strain harbouring both pBH14 and pKH46-1:: $\gamma\delta$  survived as well in serum as did a strain harbouring only pKH46-1:: $\gamma\delta$ . All derivatives of KH933 grew equally well in rabbit serum in which complement had been inactivated by heating to 56 °C for 30 min.

The effects of ColV derivatives on the survival of bacteria in serum are therefore correlated with their effects on pathogenicity; only plasmids which greatly increased the survival of KH933 in serum enhanced the pathogenicity of the strain for chicks.

Both pBR322:*Hind*III-B and pBR322:*Bam*HI-A increased the survival of KH933 in serum, so the *iss*<sup>+</sup> determinant maps within the 5.3-kilobase sequence at 19.0–24.3 kilobases in pKH46-1:: $\gamma\delta$ . Within this region, neither the 19.0–21.0-kilobase sequence nor the 22.6–24.3-kilobase sequence encodes all the information required for expression of the *iss*<sup>+</sup> determinant, as neither pBH11 (containing the 22.6–30.7 kilobase fragment) nor pBR322:*Eco*RI-A greatly increased survival in serum. In addition, introduction of the Tn5 transposon at the 19.9-kilobase position does not affect the serum resistance or the increased virulence conferred by pKH46-1:: $\gamma\delta$ .

Seven of the ColV derivatives were also examined for their effects on the survival of *E. coli* K-12 in serum. All *E. coli* K-12 strains harbouring derivatives of pKH46-1:: $\gamma\delta$  were killed much more rapidly by fresh rabbit serum than were derivatives of KH933. However, plasmids which increased the pathogenicity and the serum resistance of KH933 also increased the survival of *E. coli* K-12 (strain W3110). The survival of *E. coli* K-12 strains was tested as described in Fig. 2 legend, except that bacteria were added to serum at an initial concentration of 10<sup>7</sup> ml<sup>-1</sup>. All the following plasmids produced a similar increase in the survival of W3110 in fresh rabbit serum: pKH46-1:: $\gamma\delta$ , pKH46-1:: $\gamma\delta$  19.9kb::Tn5, pKH46-1:: $\gamma\delta$  19.9kb::Tn5 *cva*-1. None of the following plasmids increased the survival of *E. coli* K-12 in serum: pKH46-1:: $\gamma\delta$   $\Delta$  (7.4–25.6 kilobases), pKH46-1:: $\gamma\delta$   $\Delta$  (7.4–29.3 kilobases), pBH11, pBH14. For example, the



**Fig. 2** Survival of bacteria in serum. Cells from exponentially growing broth cultures at  $A_{400} = 0.4$  were collected by centrifugation and resuspended in an equal volume of 0.85% (w/v) NaCl. The suspension was diluted 100-fold in 0.85% (w/v) NaCl and then 10-fold in fresh rabbit serum. Rabbit serum was kept at  $-20^{\circ}\text{C}$  after collection and was used within 18 h. All strains were tested simultaneously using the same batch of serum. The suspension of bacteria in serum was incubated at  $37^{\circ}\text{C}$  and viable counts were made at intervals by spreading 0.2-ml volumes of appropriate dilutions onto the surfaces of nutrient agar plates (Oxoid blood agar base no. 2). The value at each point is the mean  $\pm$  s.e.m. of three independent determinations using the same batch of serum. Each of the three determinations was the mean of three spread plates. The strains are KH933 (▲), and KH933 harbouring the plasmids pBR322:BamHI-A (●), pKH46-1:: $\gamma\delta$  (■) and pBH11 (▼).

fractions of strain W3110 (pKH46-1:: $\gamma\delta$ ) surviving in serum after 30 and 60 min were  $6.4 \times 10^{-2}$  and  $2.9 \times 10^{-2}$ . Comparable values for a W3110 strain which did not harbour a plasmid were  $7 \times 10^{-3}$  and  $3.1 \times 10^{-4}$ .

### Implications of findings: close linkage of genes specifying colicin V and virulence

The correlation between the increased pathogenicity and the increased serum resistance specified by the ColV derivatives examined here strongly suggests that the primary reason for the increased virulence of bacteria harbouring ColV plasmids is that they are less sensitive to host defence mechanisms dependent on antibody and complement. The determinants for serum resistance and for colicin V are closely linked in ColV, I-K94; the *iss*<sup>+</sup> determinant maps within the pKH46-1:: $\gamma\delta$  19.0–24.3-kilobase sequence and the genes specifying colicin V and immunity to colicin V map within the 25.6–29.2-kilobase sequence (Fig. 1).

The presence of a ColV plasmid which increases virulence is not, of course, the only factor which is required to convert an otherwise benign strain of *E. coli* (such as *E. coli* K-12) into an invasive strain. Nonetheless, the high frequency of colicin V producers among *E. coli* strains responsible for recent cases of generalised infections in livestock<sup>4</sup> suggests that it is an important factor. The occurrence of the virulence determinant on plasmids specifying colicin V is not confined to recently isolated plasmids from animal sources. The ColV, I-K94 plasmid used here was isolated from a patient in 1948. The first colicin to be investigated, by Gratia<sup>26</sup>, was produced by a strain called *E. coli* V because of its virulence for rabbits and guinea pigs (the strain was isolated from the blood of an infected

rabbit), suggesting that the ColV-CA7 plasmid present in *E. coli* V also has the virulence determinant.

The frequent occurrence of the genes for colicin V and for virulence on the same plasmid, and their close linkage in ColV, I-K94, suggest that they may interact to their mutual selective advantage. The nature of any such interaction is, however, obscure, as is the ecological significance of colicins<sup>8</sup>. Several ColV plasmids increase the ability of *E. coli* strains to survive in the human alimentary tract<sup>4,5</sup>, but this does not result from the bactericidal effects of colicin V<sup>5</sup>. It may be another effect of the *iss*<sup>+</sup> determinant. The association between the genes specifying colicin V and virulence in ColV plasmids resembles the relationship between genes specifying raffinose utilisation and K88 antigen, in that almost all plasmids specifying K88 antigen, which aids colonisation of the small intestine by enteropathogenic strains, also specify raffinose utilisation, which is apparently unrelated to virulence<sup>27</sup>. However, these two genetic determinants are not closely linked in at least one plasmid; in pRI8801 they are about 30 kilobases apart<sup>28</sup>.

Whatever the reason for the relationship between the genes specifying colicin V and virulence on ColV plasmids, colicin V production may be a useful indicator of the presence of the virulence determinant in *E. coli* strains. It may be significant, for example, that colicin V is the type of colicin most frequently produced by *E. coli* strains responsible for urinary tract infections in hospitalised patients<sup>29</sup>. Other reports<sup>30</sup> indicate that colicin V may be more commonly produced by strains responsible for a variety of human extra-intestinal infections than by strains isolated from the faeces of healthy people. However, the results reported here show that the virulence determinant of ColV, I-K94 is effective in the absence of genes specifying colicin V and immunity to colicin V. It may therefore be more widespread than is indicated by the frequency of colicin V production. Plasmids which do not specify colicin V but which increase the resistance of *E. coli* to the bactericidal effects of human serum have been described (see ref. 31). It is not known whether these plasmids also increase virulence.

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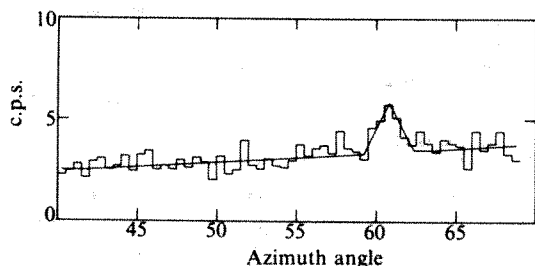
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## Soft X-ray emission from the vicinity of the dwarf nova AY Lyrae

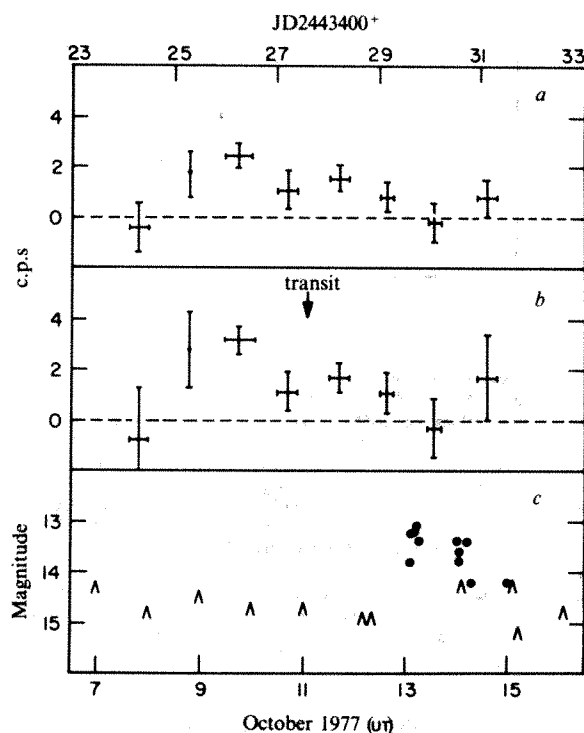
THE discovery of a low-intensity soft X-ray source in a region of the sky containing the dwarf nova AY Lyrae is reported here. Figure 1 shows how the source, designated H1839+37, appeared in the superposition of HEAO A2 scanning data that were taken on 9 October 1977 with the low-energy detector LED1. (A complete description of the A2 experiment on HEAO 1 is given by Rothschild *et al.*<sup>1</sup>.) The source was detected only in the energy interval 0.18–0.43 keV. The peak in Fig. 1 represents an intensity of  $0.014 \pm 0.0027$  counts  $\text{cm}^{-2} \text{s}^{-1}$  ( $5\sigma$ ). An upper limit to the temperature of H1839+37 was derived by comparing the ratio of the source counts in different energy bands. If a thermal bremsstrahlung spectrum is assumed,  $T_{\text{BR}} \leq 5 \times 10^6$  K, whereas a black-body fit implies  $T_{\text{BL}} \leq 1.4 \times 10^6$  K. A lower limit  $T_{\text{BL}} \geq 1 \times 10^5$  K is set by the LED1 lower-level discriminator.

The region of the sky containing H1839+37 was scanned by HEAO 1 for several days. The light curve for the entire October 1977 observation is shown in Fig. 2a. Each point is the superposition of 1–5 individual scans made on a given day. For a half day, over which the spin axis of the satellite is held fixed, an estimate of the variability of H1839+37 can be made independently of knowing the exact source position, that is, without requiring corrections for the transmission of the LED1 collimator. Applying a  $\chi^2$  test to the five 9.5–10.0 scans for October (not shown here), the source is found to be constant at only the 10% confidence level ( $\chi^2 = 7.68$  for 4 d.f.). The source is definitely variable on longer time scales because when it was observed 6 months later it was not detected, even in a superposition of 5.5 days of scanning data. The upper limit to the intensity for that observation (7–12 April 1978) is 0.005 counts  $\text{cm}^{-2} \text{s}^{-1}$ .

The centroid of the position error box for H1839+37 is at  $\alpha = 18 \text{ h } 38 \text{ min } 58 \text{ s}$ ,  $\delta = +37^\circ 52.4'$  (1950 epoch). The error box, shown in Fig. 3, excludes the bright star Vega ( $\alpha$  Lyrae).



**Fig. 1** Part of the data from a superposition of soft X-ray (0.18–0.43 keV) scans on 9 October 1977. The intensity of the source at scan angle  $61^\circ$  was determined by making a least squares fit (the smooth line) to the data. The fitting procedure took into account the triangular response of the collimator, which had a  $1.5^\circ$  field of view (FWHM) in the scan direction. The area of the detector was  $174 \text{ cm}^2$ .



**Fig. 2** a, The soft X-ray light curve of H1839+37 for October 1977. b, Same as (a), corrected for a source at the position of AY Lyr. c, The AAVSO visual light curve for AY Lyr covering the HEAO 1 observation.

Also shown is the slightly overlapping error box for 4U1852+37, a hard X-ray source with an intensity of  $0.57 \pm 0.15$  Uhuru flux units (UFU) (ref. 2 and Jones, personal communication). The HEAO A2 upper limit for hard X-ray emission from either H1839+37 or 4U1852+37 is  $1/3$  UFU (Swank, personal communication).

H1839+37 was discovered in a survey of  $\sim 130$  cataclysmic variable stars<sup>3</sup>; it corresponds to the position of one of the members of this class—the dwarf nova AY Lyrae. Three other dwarf novae have been identified with soft X-ray sources: SS Cygni, EX Hydrae, and U Geminorum. This, plus the fact that a search in the H1839+37 error box has revealed no other promising candidates, make AY Lyr seem like a possible counterpart for H1839+37.

AY Lyr belongs to the subset of dwarf novae that have both normal outbursts and super outbursts<sup>4</sup>. Patterson<sup>4</sup> reports that the quiescent visual brightness of the system is  $V > 18$ . At the time of the HEAO 1 sighting on 9 October 1977, AY Lyr was at  $V > 14.8$  (Mattei, personal communication). Assuming a flat response across the LED1 bandwidth (0.18–0.43 keV), the 0.25 keV flux from a source at the position of AY Lyr is  $\sim 1 \times 10^{-11} \text{ erg cm}^{-2} \text{s}^{-1}$ . The ratio of the X-ray to optical flux for the other dwarf novae in quiescence is  $\leq 1$  (ref. 3). For AY Lyr, this ratio does not exceed unity only if the dwarf nova was as

bright as  $V \sim 15$  during the HEAO 1 observation. In Fig. 2b the October 1977 light curve of H1839+37 has been corrected for a source at the position of AY Lyr, taking into account the transmission of the LED1 collimator. A comparison of the X-ray light curve with the AAVSO visual light curve for AY Lyr, which is shown in Fig. 2c, reveals that there was no increase in the X-ray activity during a short optical outburst of the dwarf nova<sup>5</sup>. There were only three LED1 scans during the October outburst; these occurred 0.4–0.6 d after the beginning of the outburst. A short visual outburst also occurred on 9 April 1978 when AY Lyr was again being scanned by HEAO 1 (ref. 5), but no X rays were detected from the object at that time either.

Part of the difficulty in interpreting this observation is in not having a good measurement of the X-ray spectrum of H1839+37. The dwarf novae detected as X-ray sources seem to have two very different spectral components: a medium or hard X-ray ( $E > 0.5$  keV) component which fits a thermal bremsstrahlung model with line emission<sup>6</sup>, and a soft X-ray ( $E < 0.5$  keV) component whose spectrum may fit any of several models<sup>3</sup>. The relative intensities of both components are correlated with the dwarf nova optical outburst. The soft component increases with increasing visual brightness, but the optically thin component is not uniquely correlated: in SS Cyg this component dramatically decreases during outburst<sup>7</sup>, while in U Gem the converse is observed<sup>8</sup>. In addition, the range of temperatures for the 'hard' component spans an order of magnitude: from  $\sim 20$  keV for SS Cyg to  $\sim 5$  keV for EX Hya and U Gem (refs 6 and 8). Therefore, with so few examples and such a wide range of behaviour, it is not difficult to envisage a behaviour pattern for H1839+37 which is consistent with the X-ray behaviour of dwarf novae.

For example, in AY Lyr variable accretion onto a white dwarf primary could account for X-ray flaring during quiescence. If the spectrum were optically thin, then absorption by increased in-falling material during outburst could produce a decrease in the bremsstrahlung X rays (an effect similar to that observed in SS Cyg during outburst). The bright soft X-ray excess observed in SS Cyg during outburst may not appear in AY Lyr because the boundary layer between the white dwarf and the compact star in AY Lyr may not get hot enough to radiate soft X rays. Differences in mass accretion rates during quiescence and outburst among all the dwarf novae may account for the range of observed behaviour.

Our intention has been simply to point out a correspondence in the positions of the soft X-ray emitter H1839+37 and the

dwarf nova AY Lyr, and to present all the pertinent data on both the X-ray and optical sources. Note that a search for a set of 130 randomly distributed error boxes ( $\sim 0.6^\circ \times 6^\circ$  in size), such as the cataclysmic variable survey, would have about a 40% chance of producing a positional coincidence with one or more of the  $\sim 50$  soft X-ray sources in the HEAO A2 Catalogue (in preparation). Thus there was a relatively high expectation of finding one fortuitous association of an X-ray source with a cataclysmic variable. If H1839+37 has a 'steady' emission even a factor of 10 below the HEAO 1 threshold, the X-ray telescope on the recently launched Einstein Observatory (HEAO 2) should be able to locate it accurately.

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## Drift rates of Jupiter's S-bursts

THE average drift rates of Jupiter's S-bursts observed in the 21–23 MHz frequency range depend on the jovian declination of the Earth,  $D_E$  (ref. 1). The fact that the values obtained by other observers at higher and lower frequencies also seemed to agree with this observation was interpreted as either being coincidental or implying that the drift rates may not depend on frequency. Here I present new drift rate measurements at 21–23 MHz obtained in 1978, bringing the total number of individual measurements in 1974–78 to over 12,000.

The dynamic spectra of bursts were recorded on 16-mm film in real time with a 48-channel radio spectrograph. The S-spectra show a wide variety of forms<sup>2</sup>. The simplest bursts appear as lines tilted in the orthogonal time-frequency plane. Samples of such events, from which drift rates are measured, are shown in Fig. 1. The samples illustrate the fact that the instantaneous bandwidth of bursts can vary considerably while the drift rate remains essentially the same.

All the simple bursts are observed to have a negative frequency drift. The rate varies from burst to burst and from group to group. The spread in rates within a storm is, as a rule, relatively large. The average drift rates do not seem to be related to the central meridian longitude (CML) of Jupiter or to Io's geocentric phase<sup>1,2</sup>. The instantaneous bandwidths, however, tend to be larger at the high-longitude edges of region B. The S-bursts occur only in the Io-related regions B and C (refs 2, 3).

The mean drift rates for B-region storms observed in 1974–78 are plotted as a function of  $D_E$  in Fig. 2. As can be seen, the rates change from approximately  $-30 \text{ MHz s}^{-1}$  to  $-20 \text{ MHz s}^{-1}$  as  $D_E$  decreases from  $3.3^\circ$  to  $0.5^\circ$ . It is now obvious that the drift rates in the vicinity of  $-10 \text{ MHz s}^{-1}$  which were obtained at other low values of  $D_E$  (refs 1, 4) must have been due to the lower observation frequency used, suggesting that the drift rates of S-bursts in region B are proportional to both  $D_E$  and frequency.

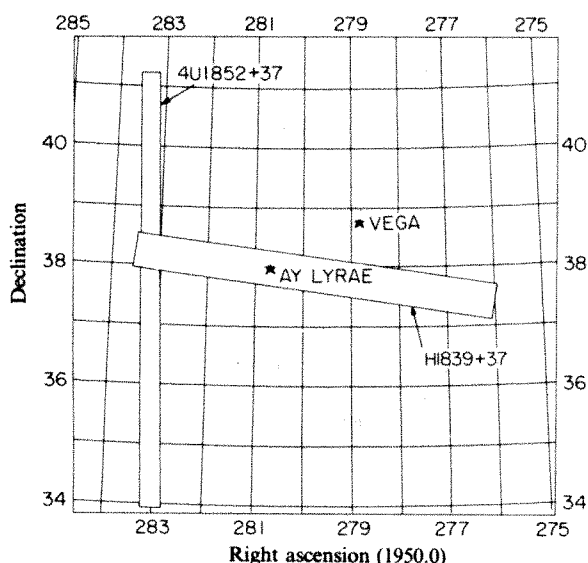
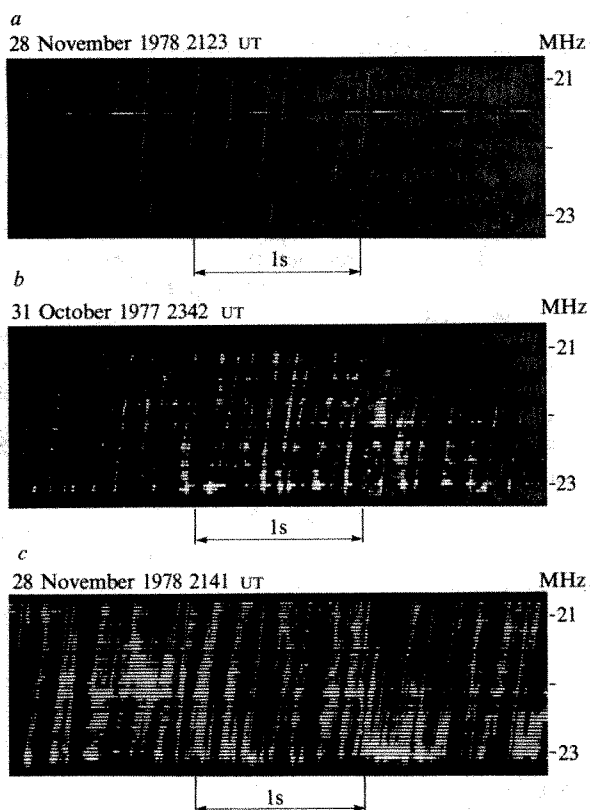
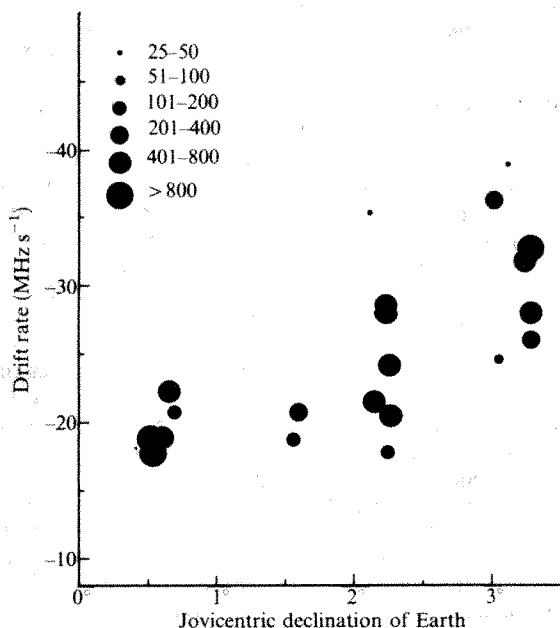


Fig. 3 H1839+37 error box (90% confidence), also showing the positions of Vega, the dwarf nova AY Lyr, and the error box for a 4U source.



**Fig. 1** Samples of high-resolution dynamic spectra of jovian S-bursts. Time increases from left to right. The instantaneous bandwidths of the bursts in *a* are 50 kHz or less, the bandwidths of certain bursts in *b* are a few hundred kHz, and those in *c* vary from 50 kHz to 1 MHz.



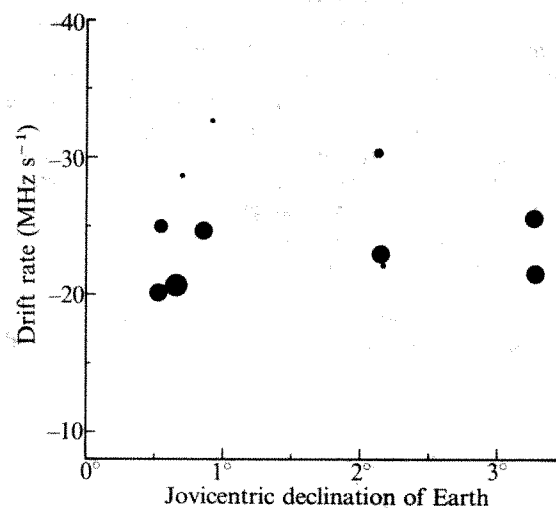
**Fig. 2** Mean drift rates of S-bursts observed in storms of region B, plotted as a function of the jovicentric declination of the Earth. Each circle represents one storm, its size denoting the number of measurements.

If the mean drift rates of S-bursts in region C are plotted as a function of  $D_E$ , no clear dependence on  $D_E$  is evident. This is illustrated in Fig. 3, comprising storms observed in 1976 and 1978.

In a model often referred to, the sources of S-bursts move outwards from Jupiter along the magnetic field lines within the Io flux tube<sup>4-6</sup>. The frequency of emission is close to the local electron gyrofrequency, while the frequency drift is determined by the longitudinal velocity component of the source. The  $D_E$ -effect can be explained by assuming that the emission is sharply beamed so that a relatively small change in  $D_E$  may lead to a selection of bursts having different drift rates and different emission angles.

A similar selection effect should also occur due to the variations in the CML and the geocentric phase of Io. According to the Earth-Jupiter viewing geometry<sup>7</sup>, the viewing angle of an Io-threaded field line varies as a function of the CML and Io phase more than it does as a function of  $D_E$ . The storm-averaged drift rates, however, show no significant dependence on the CML or Io phase. Thus the evidence suggests that the explanation of the  $D_E$ -effect based on sharp beaming from upward-drifting electrons in the Io flux tube is not correct.

Another possible interpretation is that the variation of drift rates is not determined by a selection process but that the rates of all bursts vary together. In that case, a true physical relationship with the Sun may be involved, such as the direction of arrival of the solar wind, related to the jovicentric declination of the Sun,  $D_S$  (ref. 8). Since  $D_S$  never differs much from  $D_E$ , this possibility cannot be ruled out on the basis of the present series of observations covering less than half a cycle of  $D_E$ .



**Fig. 3** Diagram as in Fig. 2, but for S-bursts observed in storms of region C.

One more possibility is that neither  $D_E$  nor  $D_S$  is the controlling factor, but that the solar activity is. If the storm-averaged drift rates are plotted as a function of the averaged sunspot number, an inverse relationship is displayed (not shown). This effect may be coincidental since the periods of  $D_E$  and of the solar activity are almost the same. Arguments relating to the relationships between  $D_E$  and  $D_S$  and the sunspot number have been presented previously in studies of the occurrence probability of L-burst and the longitude drift of emission regions<sup>8,9</sup>. Many years of observation would probably be necessary to show whether the true correlation is with  $D_E$  (or  $D_S$ ) or solar activity, or neither.

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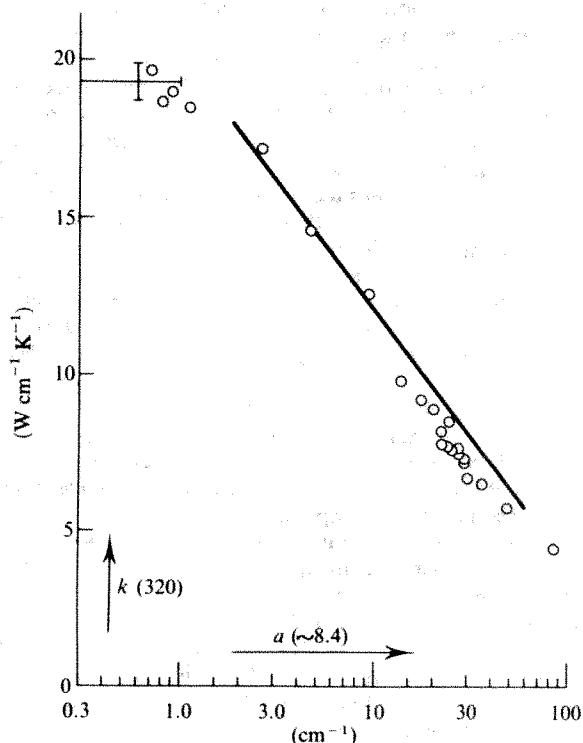
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## Thermal conductivities of diamonds with absorption at 3.22 $\mu\text{m}$

MEASUREMENTS of the thermal conductivities of a hundred diamonds at temperatures between 320 and 450 K have been reported<sup>1</sup>. These results were interpreted in terms of nitrogen impurity alone, although it was suggested that hydrogen might give an effect in other diamonds with absorption peaks at 3.22  $\mu\text{m}$  wavelength. We report here measurements of the thermal conductivities of 24 natural diamonds with this IR absorption<sup>2,3</sup>.

A variety of phonon scattering mechanisms determine the thermal conductivity of diamond as a function of temperature<sup>4-7</sup>. Phonon scattering at crystal boundaries and large defects is dominant at low temperatures, whereas point defects are mainly important above room temperature. The conductivity of type IIA diamonds is extremely high over a wide temperature range. In the IR, these diamonds generally show only the fundamental lattice absorption (which is less than 1  $\text{cm}^{-1}$  above 6.5  $\mu\text{m}$ ). Additional absorption bands in type I diamonds at 7.80 and 8.30-8.55  $\mu\text{m}$  have been attributed to



**Fig. 1** Thermal conductivity at 320 K against maximum absorption at 8.30-8.55  $\mu\text{m}$ .  $\circ$ , Results for diamonds with 3.22- $\mu\text{m}$  peaks. The line represents the mean result for type I diamonds without 3.22- $\mu\text{m}$  peaks. The mean result for type IIA diamonds without 3.22- $\mu\text{m}$  peaks is shown with error bars representing 1 s.d. at upper left. Mean results from ref. 1 (Fig. 5).

**Table 1** Experimental results

Diamond no.	$k(320)$ ( $\text{W cm}^{-1} \text{K}^{-1}$ )	$k(450)$ ( $\text{W cm}^{-1} \text{K}^{-1}$ )	$p(3.22)$ ( $\text{cm}^{-1}$ )	$a(7.3)$ ( $\text{cm}^{-1}$ )	$a(7.80)$ ( $\text{cm}^{-1}$ )	$a(\sim 8.4)$ ( $\text{cm}^{-1}$ )
101	19.7	12.7	0.7	0.6	0.6	0.7
102	19.0	12.3	1.3	0.7	0.9	0.9
103	18.7	12.6	0.6	0.9	0.8	0.8
104	18.5	11.6	1.2	1.0	1.0	1.1
105	17.2	11.4	4.2	1.3	2.2	2.6
106	14.6	10.9	2.8	4.6	3.4	4.6
107	12.6	9.5	1.7	4.8	6.4	9.2
108	9.8	7.3	13.6	6.4	16.0	13.8
109	9.2	7.1	2.8	9.1	22	17.2
110	8.9	6.8	2.6	6.5	27	20
111	8.5	6.7	3.4	0.8	43	24
112	8.2	6.2	2.3	8.4	35	22
113	7.8	6.3	8.5	10.3	29	22
114	7.7	6.1	11.7	10.3	39	26
115	7.7	6.1	8.7	9.9	40	27
116	7.7	6.2	9.4	10.9	30	24
117	7.6	5.9	12.1	17.8	21	25
118	7.5	5.9	10.2	15.2	34	27
119	7.3	5.8	12.1	14.5	34	29
120	7.2	5.9	15.9	6.3	45	29
121	6.7	5.3	11.6	4.3	41	30
122	6.5	5.2	8.9	22	46	36
123	5.7	4.6	10.2	13.5	77	48
124	4.4	3.6	2.5	51	85	85

nitrogen<sup>8</sup>, which can be present in two forms in the diamond lattice<sup>9,10</sup>. For the hundred diamonds of ref. 1, it was found that the maximum absorption in the range 8.30-8.55  $\mu\text{m}$  can be used to predict the conductivity and it was concluded that nitrogen in concentrations higher than about  $5 \times 10^{-3}$  atm% gives detectable lowering of the conductivity.

The diamonds used in the present work were polished to rectangular bars free from cracks and inclusions at  $\times 20$  magnification. Experimental details of the measurements of thermal conductivity and IR absorption were reported previously<sup>1</sup>. Mean thermal conductivity values  $k$  at 320 and 450 K were derived from measurements of each diamond at these and intermediate temperatures by means of  $k \propto T^{-s}$ , where  $s$  is a constant for each diamond<sup>1</sup> (Table 1). The maximum absorption value in the range 8.30-8.55  $\mu\text{m}$  is denoted as  $a(\sim 8.4)$ . The value  $p(3.22)$  is the strength of a narrow absorption peak at this wavelength. It is defined as the peak absorption minus the fundamental lattice absorption (indicated by the trend of the absorption curve across the base of the peak). The 3.22- $\mu\text{m}$  peak occurs in combination with one at 7.12  $\mu\text{m}$ .

Diamonds 101-104 are type IIA diamonds with 3.22- $\mu\text{m}$  peaks. Such diamonds seem to be rare. Their conductivities were found to be about as high as those of type IIA diamonds without 3.22- $\mu\text{m}$  peaks. However, the 3.22- $\mu\text{m}$  peaks of diamonds 101-104 are rather small and the diamonds with larger peaks also show absorptions due to nitrogen. Analysis of the absorptions of type I diamonds 105-124 gave no correlation between the amount or ratio of the two nitrogen forms and  $p(3.22)$ . Therefore, and in view of the existence of diamonds 101-104, the nitrogen impurity level seems to be independent of the value of  $p(3.22)$ . Diamond 124 has the lowest conductivity and a very high value of  $a(\sim 8.4)$ . From these values and ref. 1 it can be inferred that this diamond contains a very high concentration of nitrogen ( $0.5 \pm 0.1$  atm%). This has been confirmed by nuclear activation analysis<sup>11</sup>.

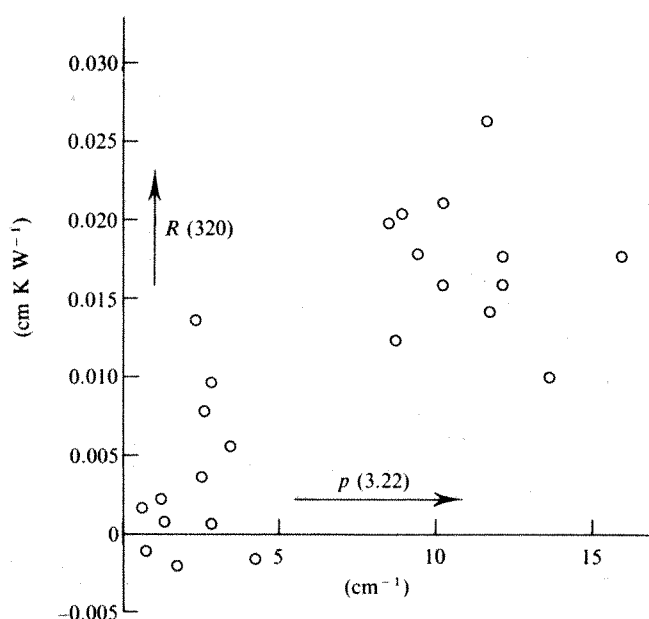
The results for the 24 diamonds are compared with the mean results for diamonds without 3.22- $\mu\text{m}$  peaks in Fig. 1. Values tend to lie below the line derived previously<sup>1</sup>. An extra thermal resistivity

$$R(320) = \frac{1}{k(320)} - \frac{1}{k_L(320)} \quad (1)$$

has been calculated for each diamond. The subscript L refers to the line in Fig. 1 or, in the case of diamonds 101-104, to the value for type IIA diamonds. The results have been plotted

against  $p(3.22)$  in Fig. 2. It can be concluded that diamonds with large  $3.22\text{-}\mu\text{m}$  peaks have a small but non-zero extra thermal resistivity. The same conclusion was reached from the data for 450 K.

This extra thermal resistivity probably arises from phonon scattering at hydrogen point defects in the diamond lattice. The presence of hydrogen impurity in some diamonds is well established, concentrations as high as 1 atm% having been reported by Sellschop *et al.*<sup>12</sup> and by Hudson and Tsong<sup>13</sup>. However, only one of Hudson and Tsong's diamonds showed the  $3.22\text{-}\mu\text{m}$  absorption. Runciman and Carter<sup>3</sup> attributed the  $3.22$  and  $7.12\text{-}\mu\text{m}$  absorptions to stretching and bending vibrations of C—H bonds and estimated the hydrogen density at a



**Fig. 2** Extra thermal resistivity at 320 K against the strength of the peak at  $3.22\text{-}\mu\text{m}$ . The accuracies of the ordinates are about  $\pm 0.01\text{ cm K W}^{-1}$  and of the abscissae  $\pm 0.3\text{ cm}^{-1}$ .

much lower level (equivalent to  $0.006\text{ atm}\%$ ), a result which they stated was confirmed by a vacuum fusion experiment. Chrenko *et al.*<sup>2</sup> also assigned these absorptions to C—H vibrations, but did not estimate the hydrogen concentration.

We believe that the larger hydrogen concentrations ( $\sim 1\text{ atm}\%$ ) are most probably associated with relatively large impurity aggregates such as magma droplets<sup>14</sup> or micro-inclusions<sup>15</sup>. These would not lower the thermal conductivity significantly above room temperature. Following Runciman and Carter<sup>3</sup> it seems that the  $3.22\text{-}\mu\text{m}$  absorption is probably associated with overall hydrogen concentrations some 2 or 3 orders of magnitude lower. These would then be the hydrogen point defect concentrations responsible for the extra thermal resistivity. This would be consistent with an extension to hydrogen defects of the analytical treatment of phonon scattering in diamond worked out by Turk and Klemens<sup>6</sup> (as used for nitrogen by Berman and Martinez<sup>7</sup>). However, the assumptions in this extension of the theory are so great that we can draw no positive support from such consistency.

We conclude that there is a small extra thermal resistivity associated with the sharp  $3.22\text{-}\mu\text{m}$  IR absorption in diamond. Both phenomena could be due to a small amount of hydrogen ( $\leq 10^{-2}\text{ atm}\%$ ) in point defect form, but such causation has not been proved.

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## Clouds and the long-term stability of the Earth's atmosphere and climate

CLOUDS dominate the albedo of the Earth and hence have a vital role in the global radiation balance. They are one of the most important physical properties of the atmosphere but the most difficult to parameterise. The forecasting of short-term climatological excursions (weather) requires numerical integration of complex dynamical models. However, over longer time periods it may be possible to include cloud cover without resorting to explicit atmospheric dynamics. Here we suggest that over evolutionary time periods ( $10^8$ – $10^9$  yr) the Earth's percentage cloud cover has remained approximately constant. This is in general agreement with present ideas about the stability of the Earth's evolution<sup>1–3</sup>. Over medium-term climatological periods ( $10^4$ – $10^7$  yr) we have found that the position of large cloud masses may be directly related to the changing surface configuration, caused, for example, by continental drift. Global cloud cover fluctuates about a mean, which is near the present-day value and reinforces albedo changes caused by surface configuration; this could be highly significant for theories of climatic change.

The high reflectivity of clouds results in their dominance of the global albedo of the Earth even though they cover, on average, only about 50% of the surface area. Recently<sup>4,5</sup> it has been demonstrated that the reflectivity in the visible wavelengths is more important than the complementary increase in IR opacity. Thus changing cloud amount cannot be ignored as had been suggested<sup>6</sup>, but must be considered as important in both long- and short-term climatic simulations. Cloud amount can be assumed to be a function of both the surface temperature and the available atmospheric water vapour. Contrary to some evolutionary models of the Earth's atmosphere<sup>7</sup>, we have found that the average global cloud amount has probably varied very little throughout the lifetime of the Earth.

Any long-term climatological model of the Earth must be concerned with calculation of the surface temperature,  $T_s$ , as we have observational data (from the geological record) for this parameter. It is generally agreed that throughout most of the planet's history<sup>2,8</sup> the value of  $T_s$  has probably not varied significantly from that of the present day. Changes in the value of  $T_s$  may cause responses within the atmosphere–hydrosphere

**Table 1** Albedo values,  $A$ , calculated for varying cloud cover

Percentage cloud cover	Albedo (%)
50 (present day)	30.1
40	24.6
60	35.7

system which could in turn perturb the global climate further. Some climate theories have developed the theme of temperature evolution via cloud changes<sup>7</sup>, although more recently<sup>1,8</sup> the generally stable nature of the Earth's evolution and long-term climate has been emphasised. Here we examine the atmospheric response to changes in the global average surface temperature. As it seems that the albedo effect of cloud is of greater importance than the absorption in the IR wavelengths, it will be changes in the albedo via cloud changes which will lead to climate perturbations. Therefore we have used a computer model to calculate the Russel-Bond spherical albedo of the Earth. This model derives a value for the global albedo,  $A$ , by integrating the reflected intensity over all points of the Earth's surface, making allowance for variations in reflectivity and angle of incidence at each point. The model has been described in detail elsewhere<sup>9</sup> and has been shown to give results consistent with measured values for  $A$ . The numerical integration includes the reflection due to clouds and is more satisfactory than calculations<sup>4,6</sup> which simply derive the global albedo from

$$A = A_c \alpha_c + A_s(1 - \alpha_c) \quad (1)$$

where  $A_c$  and  $A_s$  are the average reflectivity values of cloud and surface and  $\alpha_c$  the fractional cloud cover. Our model allows the importance and amount of both cloud and surface configuration to be estimated. The results have been used in two complementary modes below.

The major global sensitivity to changes in the surface temperature could be expected to come via the cloud response. It has recently been calculated<sup>10</sup> that, contrary to the widely held view, increasing surface temperature leads to decreased cloud cover (as a percentage of the surface area covered). Sellers<sup>11</sup> has suggested that this decrease could be caused by the build-up of convective clouds rather than stratiform clouds (that is clouds with larger vertical extent but smaller horizontal area). Several complex general circulation models are producing this type of result<sup>6,12</sup>. Roads<sup>10</sup> suggests that the cloud amount will probably decrease by 1% per degree K increase in  $T_s$ . It is now possible to investigate the atmospheric response to changes in the surface temperature (within the ranges expected from the geological evidence).

We have considered very large changes in  $T_s$  ( $\pm 10$  K) (compare the glacial/interglacial change of approximately 5 K) to illustrate the stability even in extreme conditions, but the results hold for smaller temperature changes also. If  $T_s$  were to be increased from its present value of 288 K to 298 K the resulting increased convection would lead to a decrease in the cloud amount of  $\sim 10\%$  and hence to a lower value of  $A$ . The new value of  $A$  has been calculated from the computer model (see Table 1). The average atmospheric lapse rate can be estimated from the values of the surface temperature and the temperature at the tropopause. This tropopause temperature,  $T_{\text{TOP}}$ , is approximated by

$$T_{\text{TOP}} = 2^{-1/4} T_e \quad (2)$$

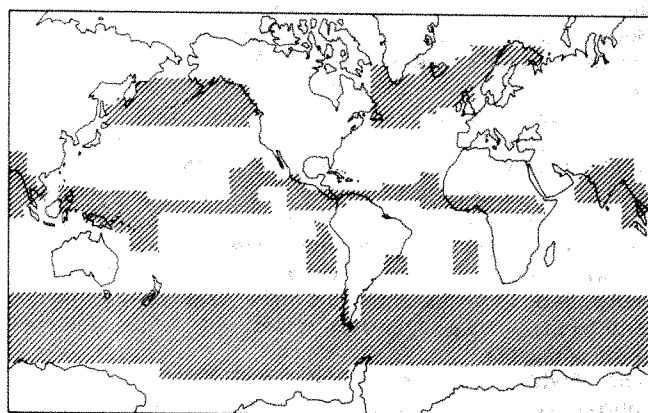
where  $T_e$  is the effective planetary temperature given by

$$\frac{S}{f}(1 - A) = \sigma T_e^4 \quad (3)$$

where  $\sigma$  is the Stefan-Boltzman constant,  $S$  the incident solar flux and  $f$  the planetary flux factor (which represents the ratio of the area of the planet emitting thermal radiation to the area intercepting solar radiation, and is a function of the planetary rotation rate and the absorption properties of the atmosphere<sup>8,13</sup>). Thus for the present-day situation we have  $T_s = 288$  K,  $T_{\text{TOP}} = 214$  K and the resulting lapse rate of between 6 and 7 K km<sup>-1</sup> (in good agreement with measured values and more complex models<sup>14</sup>).

In the surface temperature increase thesis the new value of  $T_{\text{TOP}}$  is 218 K, and thus the lapse rate increases which, in turn, is likely (on a global scale) to enhance cloud formation (negative feedback). Similarly  $T_s = 278$  K increases cloud, increases  $A$  to  $\sim 35.7\%$  (see Table 1) and gives  $T_{\text{TOP}} = 210$  K. The resulting lapse rate decrease would tend to inhibit further cloud build-up (of all types) and thus secure a return to a stable regime. Our contentions regarding the combined effect of changing the surface temperature and the atmospheric lapse rate are difficult to prove for the globally averaged situation we are discussing. However, similar arguments regarding the average atmospheric stability and the likelihood of cloud condensation have been considered for a number of planets by Goody and Walker<sup>15</sup>. Thus it appears that even large changes in  $T_s$  are unlikely to produce any catastrophic responses from the atmosphere.

The overall dynamic stability which is indicated by these results could, however, be disrupted by a secondary effect of changing cloud patterns via the possible variation in cloud positions. Obviously the cloud configuration on a global scale is a function of the general circulation. Within this framework the cloud cover may also be dependent on the predominant underlying surface type. Over very long time periods ( $10^6$ – $10^8$  yr) the relative positions of continents and oceans has changed greatly, and this may be an important factor for studies of the long-term temperature evolution. Henderson-Sellers and Meadows<sup>16</sup> have shown that continental land mass movements are of importance for albedo changes and hence may be responsible for enhancing or stimulating glacial periods. This preliminary study excluded cloud cover perturbations. The data regarding cloud distribution are still extremely sparse, and any attempt to draw comparisons between 'continental' and 'oceanic' coverage patterns at this stage must be tentative. Studies have been carried out, however, to estimate the likelihood of cloud cover of surface features for surveillance from satellites<sup>17</sup>. This study describes cloud cover in terms of climatological regions. I<sup>18</sup> have used this cloud data base to establish an objective method of cloud description using the beta probability distribution. From these studies it is possible to generate cloud probability maps (Fig. 1). These maps can show the areas most likely to be cloudy (or cloud free) over different time periods. In terms of the evolution of the global albedo certain features of Fig. 1 are particularly interesting.



**Fig. 1** Shaded regions have very high probabilities of cloud cover: July analysis (for details of analysis see ref. 18).



**Table 2** Global albedo values,  $A$ , calculated for the two extreme continental configurations examined

Clouds	Albedo (%)	
	Configuration 1	Configuration 2
No clouds	6.8	12.1
50% Cloud cover present-day distribution	30.9	29.0
50% Cloud cover extent of ITCZ reduced	29.9	—
50% Cloud cover extent of ITCZ increased	—	31.1

Configuration 1 has all the continental mass close to the equator while configuration 2 has the continents within 40° of the poles.

Note that the high cloud cover zones appear to follow the surface features (open ocean areas) as well as the general atmospheric circulation. Hence we suggest that over very long time periods the consequences of changing cloud configuration need to be assessed, in spite of the above conclusion that total cloud cover may have changed little. We have used the computer program to generate albedo values for the Earth, including both latitudinal changes in the positions of the continents and the predominantly cloudy regions. We have retained 50% cloud cover in these calculations, whilst slightly perturbing the areas of dominant cloudiness (Table 2). Both the magnitude and direction of the change in  $A$  are significant.

Note that global albedo values only are presented here. The interactions between solar radiation and the terrestrial climate are extremely complex and depend on many factors. This complex system has recently been considerably trivialised<sup>19</sup> by the suggestion that the current distribution of continental masses favours glaciation via the 'Milankovitch' orbital perturbation effect because of seasonality of temperatures (for example, the radiation distribution leading to cool northern summers and cold southern winters retaining and initiating ice cover respectively). This is contrary to the facts—climatological studies and more recent satellite data<sup>20</sup> immediately confirm that seasonal temperature change is greater in the Northern Hemisphere and that at present the Northern Hemisphere summer is warmer than the same season in the Southern Hemisphere. This is, of course, due to the global distribution of large oceanic areas and indicates that the 'Milankovitch' mechanism, if shown to be the cause of glacial epochs, operates despite the present global geography which opposes its effects in both hemispheres. Here we simply indicate the probable effect on the global radiation balance of cloud response to two extreme surface configurations.

The configurations chosen are: (1) all the land gathered closely around the equator; and (2) all the land areas within 40° of the poles. The albedo range in the absence of clouds is considerable<sup>16</sup>. However, imposition of present-day cloud reduces this difference to almost zero and reverses the ordering. We suggest from these studies that the global cloud cover could have responded to changes in the surface land/ocean configuration. We have therefore also calculated the likely global albedoes with a constant cloud cover percentage (50%) but with slight latitudinal changes in positioning of the predominantly cloudy areas. The change in  $A$  is considerable (7.2% for polar land masses, configuration 2). It is immediately clear that the postulated cloud changes re-establish the albedo difference between the two global configurations, the polar land mass case (configuration 2) still resulting in a higher value of  $A$  (although the difference between the two models is less than was previously anticipated<sup>16</sup>).

Our model calculations of the global albedo suggest two important conclusions. First, it seems that the average global cloud cover has remained approximately constant throughout the evolutionary history of the Earth's atmosphere. Second, a much smaller effect due to the reinforcement of surface forcing

of the albedo by perturbed cloud configuration is probably important in medium-term climatic change.

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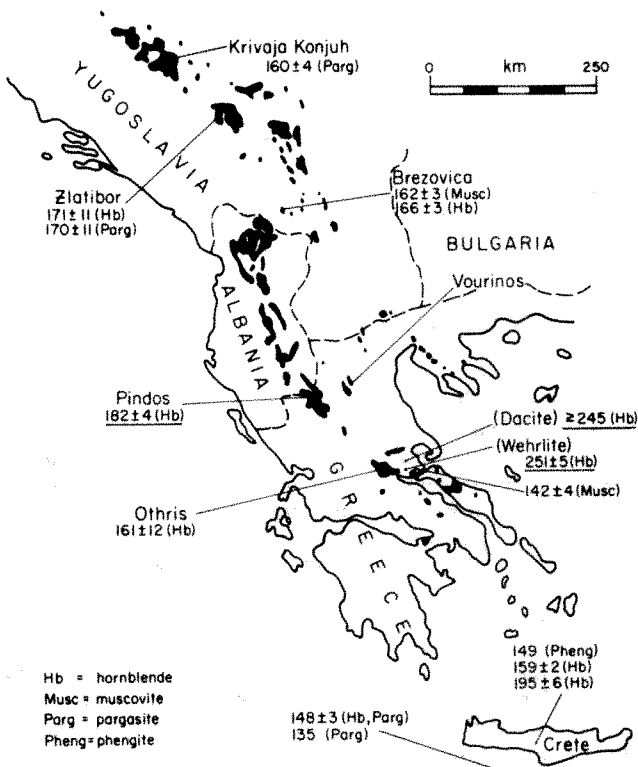
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## Permo-Triassic and Jurassic <sup>40</sup>Ar-<sup>39</sup>Ar ages from Greek ophiolites and associated rocks

PRECISE radiometric dating of ophiolites and associated rocks is usually very difficult as the rocks are often altered by ocean floor metamorphism or during tectonic emplacement onto the continental margin. Only age determinations on primary mineral phases can be considered to approximate their initial crystallisation and cooling ages. The K-Ar technique is generally used to date ophiolitic rocks, but even if primary mineral phases are analysed, there is always the uncertainty that the resulting age could have been partially reset by later thermal or tectonic events. The <sup>40</sup>Ar-<sup>39</sup>Ar step heating method is able to detect and often to correct for any partial loss of argon, and thus overcome these problems. We have determined and report here <sup>40</sup>Ar-<sup>39</sup>Ar age spectra for hornblende separates from two unaltered samples of igneous rocks that are interpreted as the initial igneous products of rifting in the Othris region of central Greece. A third hornblende has been analysed from amphibolites believed to have been formed during tectonic emplacement of the Pindos ophiolite (Fig. 1). The ages suggest that the initial rifting began in Othris in the Permo-Triassic (~248 Myr), while tectonic emplacement of the Pindos ophiolite occurred in the middle Jurassic time (~180 Myr).

The breakup of a continental area in what is now the Othris region began in early Mesozoic time<sup>1</sup>. It is believed to be contemporaneous with the formation of picrites, wehrlites, dolerites, pillow lavas, tuffs and the deposition of associated cherts and dolomites assigned to the Agrilia Formation<sup>2</sup>. The age of the Agrilia formation is not well constrained. In parts of Othris the underlying Gavriani Formation contains Upper Permian and Lower Triassic fossils, but the Gavriani/Agrilia boundary may be diachronous<sup>2</sup>. Previously determined K-Ar ages on altered whole rocks<sup>3</sup> and amphibolite related to the emplacement of the ophiolites<sup>2</sup> were considered at best to be minimum ages of crystallisation (160, 185 Myr). <sup>40</sup>Ar-<sup>39</sup>Ar analyses of primary hornblende from a wehrlite and a dacite in the igneous sequence have been obtained (Tables 1 and 2). Age spectra and associated Ca/K ratios are given in Fig. 2a and b.



**Fig. 1** Published K-Ar ages with new  $^{40}\text{Ar}$ - $^{39}\text{Ar}$  data (underlined) on ophiolites and associated rocks in Yugoslavia, Greece and Crete<sup>2,13-15</sup>. Decay constants recommended in ref. 16 used for all data, with time scale in ref. 6.

Details of analytical procedure will be given elsewhere, but duplicate analyses indicate the precision of the data.

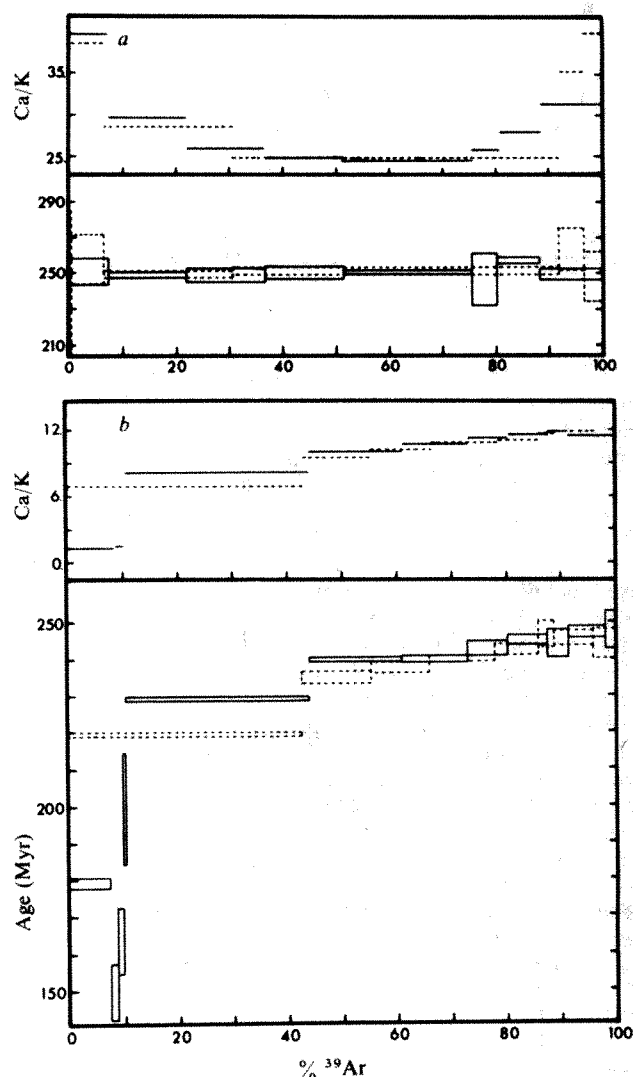
The wehrlite hornblende spectrum is flat (Fig. 2a) and indicates a crystallisation age of  $251 \pm 5$  Myr. In contrast, the dacite hornblende spectrum shows increasing age with increased

**Table 1** Sample locations and characteristics

Sample*	Locality	Description and probable significance
G-16R 108185	~2 km west of Andinita, Othris Mountains	Wehrlite: Olivine (Fo84.5) poikilitically enclosed by augite and minor brown hornblende. Part of a gabbro-peridotite cumulate sequence related to picrites. Inception of rifting and igneous activity.
G-18 108201	~3 km south-east of Dhomokos, Othris Mountains	Dacite: Phenocrysts of brown hornblende, biotite, albitised plagioclase showing relict zoning and minor quartz. The groundmass has recrystallised to fine-grained albite K-feldspar. Isolated outcrop in plain. Felsic member of the Triassic volcanic suite associated with rifting.
B76-24 123656	Avominsta valley, Perivoli	Amphibolite: Green hornblende showing strong lineation plus plagioclase. Associated with garnet-mica schists. Probably continental margin material metamorphosed by ophiolite emplacement.

\* Six-figure numbers from Harker catalogue, Department of Mineralogy and Petrology, Cambridge.

outgassing of  $^{39}\text{Ar}$  (Fig. 2b). This age spectrum approximates the diffusion loss pattern predicted by Turner<sup>4</sup> for later reheating and observed by Dallmeyer<sup>5</sup> in hornblendes in a remetamorphosed terrane. If it is assumed that the spectrum is the result of diffusive loss of the argon, then the crystallisation of the hornblende is approximated by the highest temperature step and indicates an age of  $245 \pm 5$  Myr or greater, similar to the wehrlite age. The minimum age observed in the low temperature steps suggests that the reheating took place at 150 Myr or later.



**Fig. 2** a, Age spectra for duplicate analyses of G-16 wehrlite hornblende. Integrated age is  $251 \pm 5$  Myr; 0.26% K. Error boxes ( $2\sigma$ ) in all figures are analytical only and do not include uncertainties in the irradiation parameter ( $\sim 1\%$ ). Errors on sample ages in the test and integrated ages do include this uncertainty. b, Age spectra for duplicate analyses of G-18 dacite hornblende. Integrated age is  $231 \pm 4$  Myr; 0.80% K.

These two ages of  $\sim 248$  Myr (near the Permo-Triassic boundary<sup>6</sup>) are the oldest ages so far determined on igneous rocks associated with initial rifting in central Greece. The ocean floor generated next to the continental margin cannot be older than these rocks, but it may be younger. Using the Red Sea as an analogy, Hynes<sup>1</sup> suggested that the initial igneous activity associated with rifting in the Othris region might precede truly oceanic rifting by as much as 40 Myr because of a slow rifting rate. However, Nisbet and Price<sup>7</sup>, on examining the cherts forming the upper member of the Agrilia Formation also

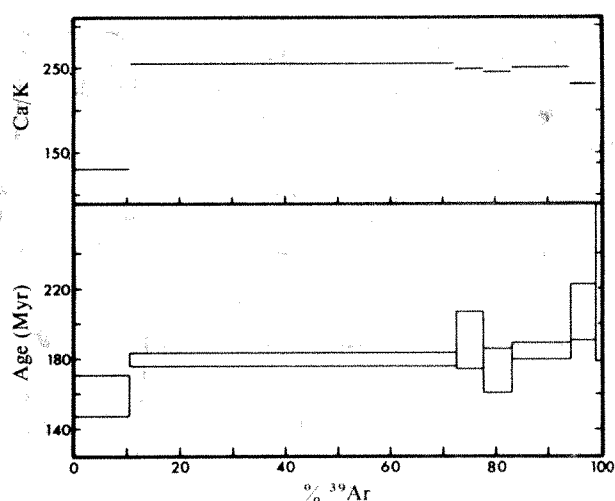


Fig. 3 Age spectra for B76-24 amphibolite hornblende. Integrated age is  $181 \pm 4$  Myr. Duplicate total fusion ages are  $186 \pm 6$  Myr and  $188 \pm 6$  Myr; 0.036% K.

suggested a slow rate of rifting ( $\sim 1 \text{ cm yr}^{-1}$ ), but they believed these cherts could have formed in an oceanic environment after only 7 Myr of rifting, resulting in an ocean basin 150 km wide. Thus a well-developed oceanic environment could have grown in a relatively short time period and the ocean floor could have an age indistinguishable within the error limits from the age of the early rifting volcanics (248 Myr). At present there are no biostratigraphic or radiometric data on the age of the ophiolites, but it is known that they were tectonically emplaced in Upper Jurassic or Lower Cretaceous time, about 135–145 Myr ago<sup>2,8</sup>. Most workers agree that not only the Othris ophiolites, but the Vourinos and Pindos ophiolites were probably emplaced at this time<sup>9,10</sup>, as well as some of the Yugoslavian examples<sup>11</sup>.

Amphibolites and other metamorphic rocks developed at the base of ophiolites are currently interpreted as having been created by the tectonic emplacement of a relatively thick and probably hot sheet of ophiolite over continental margin sequences<sup>12</sup>. Previously determined K–Ar ages on a hornblende separate and a whole-rock amphibolite from the rocks at the base of the Othris ophiolite gave average ages of 161 Myr. Because of the large analytical error ( $\pm 12$  Myr), the amphibolites did not appear significantly older than the emplacement age suggested by field evidence—about 135–145 Myr.

However, five K–Ar determinations on metamorphic rocks below three Yugoslavian ophiolites lying in the same zone as the Greek ophiolites (Fig. 1) also gave emplacement ages ranging from 160 to 171 Myr<sup>13,14</sup>.

We have analysed a hornblende separate (Fig. 3) from an amphibolite at the base of the Pindos ophiolite in northern Greece (Tables 1 and 2). Though initially emplaced in Mesozoic time, later Cenozoic thrusting has moved the ophiolite sheet so that it now lies tectonically on Eocene 'flysch'. This latest phase of emplacement has not reset the earlier Jurassic emplacement ages obtained from the amphibolites. The age spectra for 85% of the gas release is flat within the limits of analytical error and indicates an age of  $182 \pm 4$  Myr. The age clearly shows that the amphibolites have not been created by Cenozoic thrusting but are related to an earlier Jurassic period of emplacement. In Othris the ophiolite has not been moved again by Cenozoic thrusting, but the field evidence clearly indicates an Upper Jurassic or Lower Cretaceous emplacement. Thus the amphibolites in the Pindos and Othris ophiolites are significantly older than would be suspected from field evidence, but similar to radiometric emplacement ages reported from Othris and Yugoslavia<sup>2,13–15</sup>. We reconcile these data by postulating that the  $^{40}\text{Ar}$ – $^{39}\text{Ar}$  age represents the time when a hot ophiolite sheet over-rode sediments and/or basic igneous rocks on the lower continental slope, whereas the field evidence gives the age of final emplacement of the ophiolite onto a continental platform some 40 Myr later in the case of the Othris ophiolite and some 140 Myr later in the case of the Pindos ophiolite.

The most important conclusions from these data are that igneous activity associated with initial rifting in this western belt of ophiolites in Greece may date from the Permo–Triassic boundary, and that ophiolite emplacement began in the lower Jurassic. Whether emplacement was slow and steady or fast and episodic cannot be decided from the available information.

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Table 2 Mineral analyses

Mineral	G16	G18		B76-24
	(average of 4)	from	to	(average of 3)
SiO <sub>2</sub>	45.8	41.8	41.2	47.8
TiO <sub>2</sub>	4.8	4.1	3.3	0.38
Al <sub>2</sub> O <sub>3</sub>	7.9	10.5	9.4	10.9
FeO*	6.2	15.3	23.0	7.5
MnO	ND	0.22	0.49	0.14 (1 only)
MgO	16.5	11.1	6.1	15.6
CaO	11.0	10.9	10.5	12.2
Na <sub>2</sub> O	3.4	2.7	2.2	1.3
K <sub>2</sub> O	0.28	0.86	1.2	0.04†
Cr <sub>2</sub> O <sub>3</sub>	1.1	ND	ND	0.19
Total	97.0	97.5	97.4	96.1

Probe analyses by W. E. Cameron [G16, G18] and J. Spray [B76-24].

\* All iron expressed as FeO.

† Subsequent  $^{40}\text{Ar}$ – $^{39}\text{Ar}$  analysis.

ND = not determined.

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## Geological significance of a Middle Cambrian fauna from Antarctica

A NEW collection of a Cambrian fauna, occurring as erratics in a Quaternary moraine near Mount Provender (80°23'S, 29°55'W), western Shackleton Range (Fig. 1, inset) is reported here. It includes inarticulate brachiopods and trilobites, together with the first hyolithids and primitive molluscs from the area. Because of the isolation of the Shackleton Range, data on this fauna have accumulated only slowly<sup>1-4</sup>. It is important because it is located at a geographical extreme of the known extent of the Cambrian in Antarctica, and because it represents a contribution to our knowledge of world Cambrian faunas. Although the provenance of the fossiliferous erratics is problematical, it has important stratigraphical implications concerning the controversial age of the Blaiklock Glacier Group, for which ages as far apart as Cambro-Ordovician<sup>2,3</sup> and Permian<sup>1</sup> have been considered.

Other Cambrian faunas from Antarctica (Fig. 1) occur in the Transantarctic Mountains and in the Ellsworth Mountains of Lesser Antarctica. Like those of the Shackleton Range, they usually consist of assemblages of brachiopods and trilobites with or without associated archaeocyathids. In some instances exclusively archaeocyathid faunas have been found. The faunas range in age from late Lower Cambrian to Upper Cambrian<sup>5-9</sup>, although associated acritarchs at one locality in northern Victoria Land (Fig. 1, locality 9) suggest a possible brachiopod fauna might be as old as latest Precambrian<sup>10</sup>.

The Shackleton Range fauna occurs in fissile shales and siltstones. With the exception of the inarticulate brachiopods, which have shell material preserved, the fauna is present as internal and external moulds of moderate relief. In general, the brachiopods are preserved as unbroken but separated valves, the trilobites as disarticulated fragments, and the hyolithids as isolated conches. There are, however, moulds of completely articulated trilobite exoskeletons and broken brachiopod shell debris. The primitive molluscs are known from a single bedding plane on a small slab of shale; they are entire but laterally flattened.

All the brachiopods are identifiable with the obolids previously described from the area<sup>3</sup>. Two forms can be distinguished, one with thin smooth shells and the other with thicker shells having rows of concentric pits on the internal surfaces. Whether these represent two species or variants of the same species is uncertain.

The primitive mollusc is similar to some forms of *Helcionella* but is probably closer to *Mellopegma*<sup>11</sup>, a possible intermediate between the Monoplacophora and Rostroconcha. The specimens are small (the largest is 3.3 mm long), laterally compressed cap-shaped shells with strongly recurved apices and coarse concentric growth corrugations.

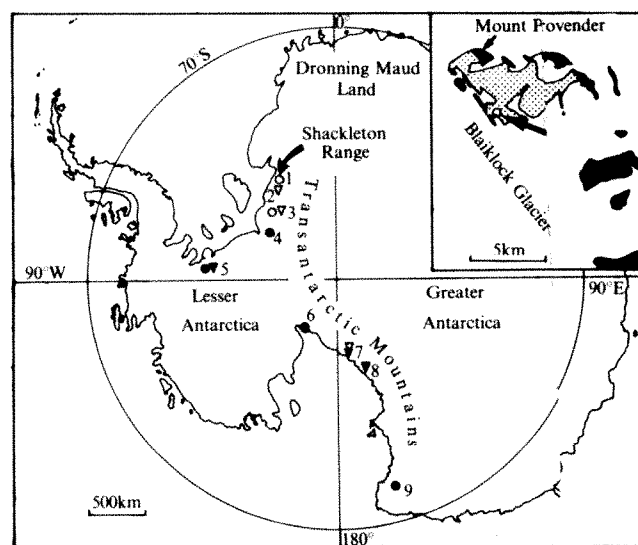
The trilobites include agnostids of *Triplagnostus* and *Peronopsis* types, and ptychoparids of *Ehmania*, *Lyraspis* and *Elrathina* types. As pointed out by Soloviev and Grikurov<sup>4</sup> these indicate a Middle Cambrian age for the fauna. The mollusc, if correctly assigned to *Mellopegma*, is also of stratigraphical value because its only previously reported occurrence is in the early Middle Cambrian of Australia. However, Runnegar and Jell<sup>11</sup> argued that morphologically similar forms must have existed as early as the Tommotian (early Cambrian).

Specimens in our collection suggest that, although the brachiopods and trilobites sometimes occur in mixed assemblages, they lived largely in different conditions. In general, the brachiopods occur in the siltstones or fine sandstones, whereas the trilobites occur more frequently in the shales. This, and the occurrence of fragmented shell accumulations, suggest that the brachiopods inhabited a slightly higher energy environment, periodically subjected to current action and reworking<sup>3</sup>.

The trilobite faunas of the Heritage, Neptune and Argentinia Ranges and Harold Byrd Mountains all occur in limestones, although associated Cambrian successions also contain clastic rocks. Together with occurrences of archaeocyathid limestones it seems that a belt of intermittent limestone deposition was present along the site of the Transantarctic Mountains in Cambrian times. By contrast, the trilobites of the Shackleton Range occur in non-calcareous shales and siltstones. In this respect they resemble the upper Middle Cambrian fauna of the Mariner Group, northern Victoria Land<sup>9</sup>, where trilobites are present in fissile mudstones attributed on sedimentological criteria to an open marine environment<sup>12</sup>. However, all known Antarctic Cambrian trilobite faunas are dominated by non-agnostids, a feature ascribed by Palmer<sup>13,14</sup> to faunas whose access to the open sea was restricted. Only the *Aphelaspis*-rich fauna of the Ellsworth Mountains<sup>8</sup> has apparently been interpreted as having had unrestricted access to the open sea<sup>13</sup>.

The source of the fossiliferous erratics is unknown but all previous geologists who have worked in the area have considered that they have not moved far. However, it is difficult to support the suggestion that the source underlies the Blaiklock Glacier Group<sup>4</sup>, presumably in pockets. Wherever the base of the Blaiklock Glacier Group is exposed it rests unconformably on the basement rocks and leaves no room for intervening sedimentary beds. Also, no evidence of the fossiliferous material, as derived blocks, has been found in the rudaceous rocks of the Blaiklock Glacier Group and the latter are obviously derived directly from the underlying basement rocks. Therefore, the original suggestion by Stephenson<sup>1</sup> that the source of the fossiliferous erratics may be intermediate strata of the group hidden beneath Blaiklock Glacier seems more probable and supposes a marine transgression following deposition of the lower terrestrial beds, and preceding rejuvenation of the landscape with subsequent deltaic sedimentation<sup>15</sup>.

Radiometric Rb-Sr age data on basement rocks in this area range from  $519 \pm 15$  Myr to  $656 \pm 66$  Myr (E. S. Grew and M. Halpern, unpublished). Compared with the Middle Cambrian



**Fig. 1** Distribution of Cambrian faunas in Antarctica. Circles denote shelly faunas, triangles denote archaeocyathid faunas (solid symbol = *in situ*, open symbol = moraine). 1, Mount Provender, Shackleton Range (Middle Cambrian); 2, Whichaway Nunataks (Lower Cambrian)<sup>5</sup>; 3, Argentina Range, Pensacola Mountains (Lower and Middle Cambrian)<sup>7</sup>; 4, Neptune Range, Pensacola Mountains (Middle Cambrian)<sup>7</sup>; 5, Heritage Range, Ellsworth Mountains (Upper Cambrian)<sup>8</sup>; 6, Harold Byrd Mountains (Middle Cambrian)<sup>6</sup>; 7, Beardmore Glacier area (Lower Cambrian)<sup>5</sup>; 8, Nimrod Glacier area (Lower Cambrian)<sup>5</sup>; 9, Evans Nève, northern Victoria Land (Lower and Upper Cambrian)<sup>9,10</sup>. The inset shows the Mount Provender area of the Shackleton Range and the collecting locality of the specimens discussed here (bold arrow). Solid areas are rock outcrop, stippled areas are moraine.

palaeontological age for the fossils, this indicates fairly rapid uplift and erosion to expose the grade of metamorphism immediately underlying the conglomerates at the base of the Blaiklock Glacier Group. These conglomerates are almost certainly the products of that uplift and erosion.

If the fossiliferous material is very local in origin it can be concluded from the limited evidence available that the lower part of the Blaiklock Glacier Group pre-dates the fossiliferous erratics so that it is no younger than Middle Cambrian. An upper age limit for the upper part of the Blaiklock Glacier Group of pre-late Carboniferous is indicated by a dolerite dyke, intruding an outlier of the upper (deltaic) part of the group at The Dragons Back, 25 km to the east<sup>2,15</sup>, which has a K-Ar age of  $297 \pm 12$  Myr (ref. 16).

The occurrence of a slab with rounded edges (about 1.5 m in diameter by 0.4 m thick) in the moraine with one surface covered in broken brachiopod shells (P. D. Marsh, personal communication) suggests a third possibility, that the fossiliferous slabs are disintegrated remnants of large glacial erratic blocks from a distant source. However, no other exotic erratics have been found in the moraine so that it is still more likely that the debris has not moved very far.

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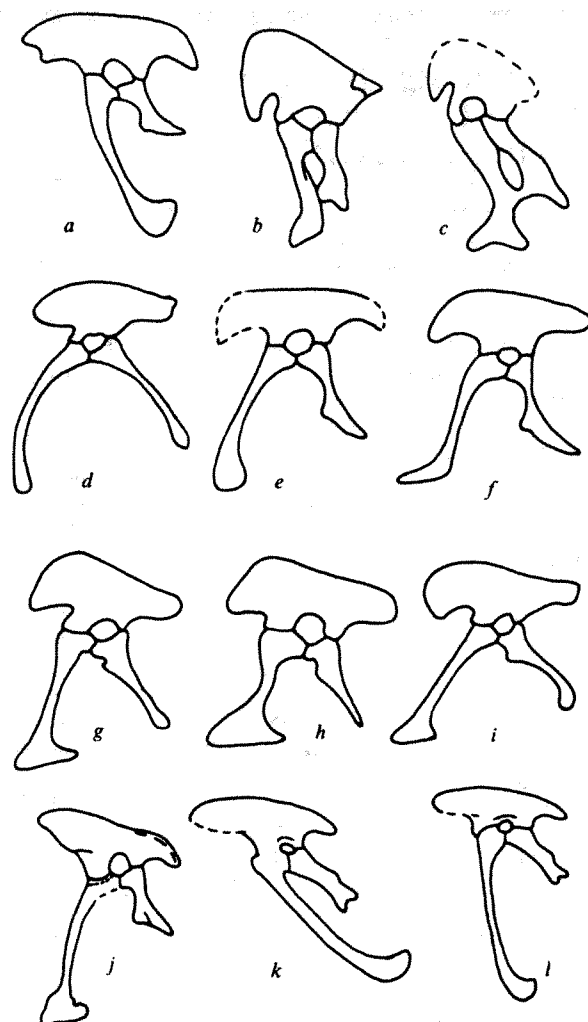
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## Opisthopubic pelvis in the carnivorous dinosaurs

THE dinosaurs are subdivided into Saurischia and Ornithischia on the basis of their pelvic girdles<sup>1</sup>, which differ chiefly in the position of the pubis. In saurischians the pubis extends forward and downward whereas in ornithischians the supposed homologue of the pubis—the postpubis—is directed backward and downward parallel to the ischium. New finds from Mongolia, however, show that some carnivorous dinosaurs had a pubis orientated parallel to the ischium. As I report here, such a pubis has been found in the dromaeosaurids<sup>2</sup>, the segnosaurs<sup>3</sup> and a new genus from the Khara Khutul locality in



**Fig. 1** Pelves of the carnivorous dinosaurs. *a*, Pelvis in the Mongolian dromaeosaurids; the same in: *b*, segnosaurs; *c*, dinosaur from Khara Khutul; *d*, podokesaurids; *e*, saurornithomimids; *f*, oviraptorids; *g*, allosaurids; *h*, Mongolian tyrannosaurids; *i*, ornithomimids; *j*, American dromaeosaurids (*Deinonychus*) as reconstructed by Ostrom (the pubis seems to be incompletely turned back); *k*, *Archaeopteryx* as traditionally reconstructed from Berlin specimen; *l*, *Archaeopteryx* as reconstructed by Ostrom (the pubis seems to be incompletely turned back as well in *Deinonychus*). The drawings in *b*, *d*, *g*, *j*, *k* and *l* are taken from refs 3, 9, 14 and 16.

south-east Mongolia, which has not yet been described and possibly represents a new family.

Dromaeosauridae<sup>4-6</sup> are known from the Cretaceous of North America and Central Asia. In the Mongolian representatives of the family, *Velociraptor*<sup>5,7</sup> and two genera not yet described, the pubis is directed backward<sup>8</sup>. In the North American *Deinonychus*<sup>6</sup> the pelvis is not fully preserved and according to the most recent reconstruction<sup>9</sup>, the pubis seems to be incompletely turned back (Fig. 1*j*). The typical dromaeosaurid pelvis<sup>2</sup> (Fig. 1*a*) is characterised by a shallow ilium, a pubis with a broadly oval and laterally flattened distal end and an ischium significantly shorter than the pubis, with a large obturator process. Thus the dromaeosaurid pelvis has the general characteristics of the small theropods and only the direction of the pubis distinguishes it from the saurischian pattern (Fig. 1*d-l*).

The ilia in the segnosaurs (Fig. 1*b*) are well separated from each other and extremely deep, with very large anterior wings strongly deflected outward. The dorsal edge of the very short posterior iliac wing has a large cubic projection. It is not clear whether this projection should be considered homologous to the antitrochanter of ornithischians or whether it represents a new structure. The re-orientated pubis has a laterally flattened, large distal end; the ischium is elongated, its obturator process being

placed more distally than in dromaeosaurids and some other small theropods.

The preserved bases of the ilia of the Khara Khutul specimen (Fig. 1c) clearly show that these were also broadly separated and had cubic projections as in the segnosaurids. However, the pubis with the boot-like distal end is quite distinct from that in segnosaurids. The obturator process is situated distally, narrow and firmly coalesced with the pubic shaft, just above the distal end of the latter. Besides some common characteristics, the pelvises of the segnosaurids and the Khara Khutul specimen each have distinguishing features.

Thus, alongside dinosaurs with a typical saurischian pelvis, lived others with a pelvis not strictly 'saurischian'. I propose to refer to the former as 'prepubic' and the latter as 'opisthopubic', although these terms have already been used to designate two main pelvic structures in saurischians and ornithischians, respectively<sup>10</sup>. The opisthopubic pelvis probably provides evidence of evolutionary changes in the function and effectiveness of several pelvico-femoral muscles<sup>11</sup>.

There is considerable similarity between the opisthopubic pelvis (particularly of the dromaeosaurids) and the primitive ornithischian<sup>12</sup> pelvis, reflecting to some extent the relationships of the two main types of pelvis and confirming the unity of the dinosaur stock<sup>13</sup>. On the other hand, the existence of the opisthopubic pelvis supports the idea that the parallel evolutionary lines of carnivorous dinosaurs<sup>2</sup> included some that transcended the limits of theropod evolution, within which predatory habits and full bipedalism<sup>14</sup> were most effectively realised on the basis of a prepubic pelvis. The opisthopubic pelvis was evidently associated with specific lines of theropod evolution<sup>11</sup>. Furthermore, the existence of this type of pelvis makes it more likely that *Archaeopteryx* had a posteriorly directed pubis<sup>15,16</sup>.

In general, the opisthopubic pelvis must have had many advantages and probably appeared several times during both the early evolution of the dinosaurs and the ancestry of birds.

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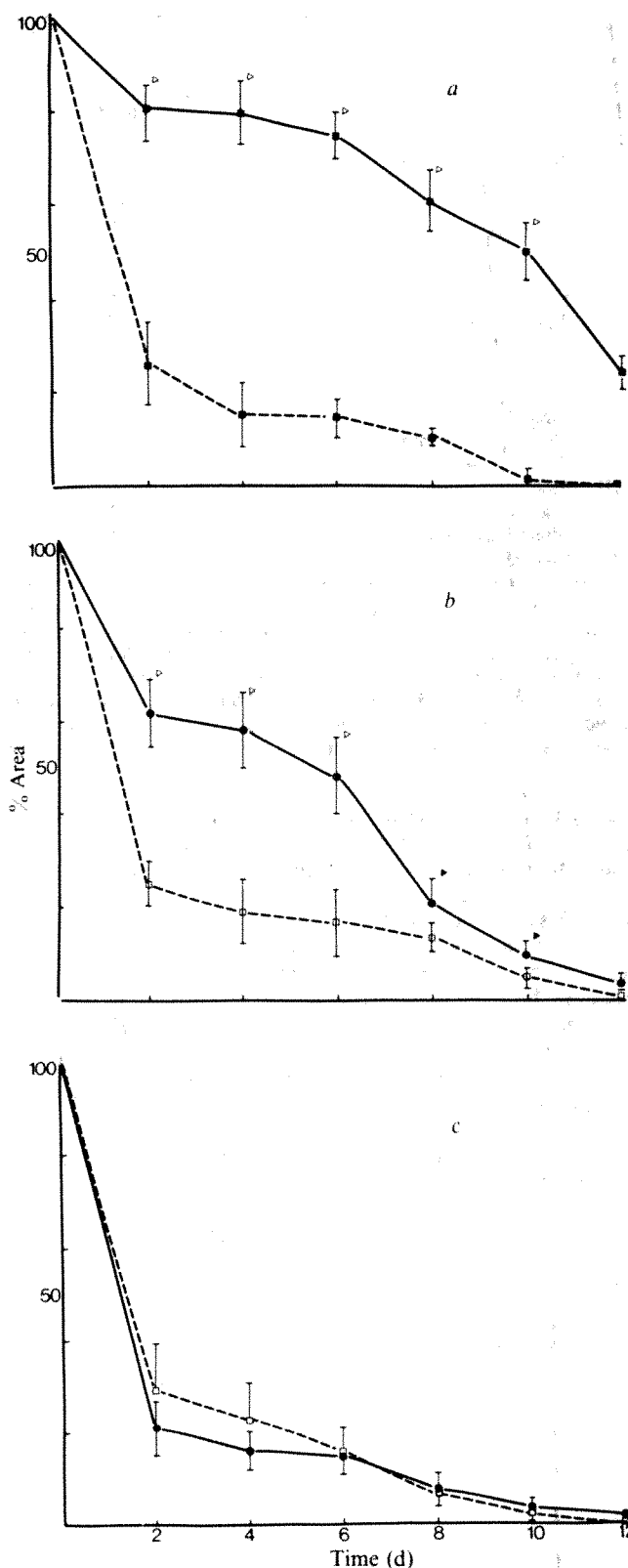
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## Effect of salivary glands on wound contraction in mice

ALTHOUGH there has been speculation about the healing powers of saliva, recent interest in the salivary glands has been concerned with the physiology of nerve growth factor and epidermal growth factor<sup>1–4</sup>. However, saliva also contains other biologically active substances such as kallikrein<sup>5,6</sup>, amylase<sup>7</sup>, lysozyme<sup>8</sup>, immunoglobulins<sup>9</sup> and renin<sup>10</sup>. Lysozyme is believed to enhance healing by suppressing infection<sup>11</sup>. The wound healing process can be divided into epithelialisation, contraction, collagen synthesis and scar remodelling<sup>12</sup>; the evidence presented here suggests that contraction is affected by the salivary glands.



**Fig. 1** Rate of subpannicular wound healing in normal mice caged separately or together (a), or after sialectomy (b, c). Results are expressed as mean % area  $\pm$  1 s.d. for five to six mice. a, Normal animals were caged either separately (■—■) or in groups (□---□). The healing rate was significantly faster with communal caging ( $\Delta$ ,  $P < 0.001$ ). b, Mice caged in groups but with prior sialectomy (SMG, SLG) (●—●) or sham sialectomy (□---□). The rate of healing in sham-treated animals was the same as in non-operated controls (Table 1bII), but was significantly faster than in sialectomised animals ( $\Delta$ ,  $P < 0.001$ ;  $\blacktriangleright$ ,  $P < 0.05$ ). c, Animals caged in groups with sialectomised (SMG, SLG) mice in the same box as sham-treated animals. In this situation no differences were found in healing rate ( $P < 0.001$ ).



**Table 1** The effect of various operative and non-operative procedures on the rate of wound healing

Experimental groups		No. of animals	No. of days after wounding					
			2	4	6	8	10	12
<b>a, Suprapannicular wounds</b>								
I	Controls (separated)	4	92±2	81±10	65±17	55±19	24±7	8±6
II	Controls (together)	10	37±15	25±8	19±7	10±2	4±2	2±1
III	Sham sialectomy	7	39±7	26±5	19±5	11±4	4±1	1±1
IV	Sialectomy (SMG, SLG)	15	96±15	78±14	58±13	30±9	12±5	5±1
V	Sialectomy (SMG)	5	55±6	48±8	32±9	19±3	14±4	2±2
VI	Sialectomy (parotid)	5	47±6	39±2	21±4	13±3	6±3	1±1
VII	Duct ligation (no delay)	6	79±15	59±11	49±6	18±6	9±9	3±3
VIII	Duct ligation (4-day delay)	6	64±7	56±9	42±5	22±5	7±2	3±2
IX	Duct ligation (7-day delay)	7	70±4	64±5	53±3	27±4	—	5±1
<b>b, Subpannicular wounds</b>								
I	Controls (separated)	6	81±5	80±7	75±5	61±7	50±6	23±4
II	Controls (together)	5	26±9	15±7	14±4	10±2	1±2	0
III	Sham sialectomy	5	25±5	19±7	17±7	14±3	5±2	1±2
IV	Sialectomy (SMG, SLG)	5	62±7	58±8	48±8	21±5	10±3	3±2
V	Shams (with sialectomies)	6	29±10	23±8	16±5	7±3	2±2	0
VI	Sialectomies (with shams)	6	21±6	16±4	15±4	8±3	3±2	1±1

The wound areas were traced on to paper using a transparent plastic sheet; the paper was cut and then weighed on a Mettler H54 balance. The weight of the tracing taken 2 h after wounding was arbitrarily designated as 100, and all subsequent measurements were expressed as a percentage. All sheets of paper came from the same batch and were of uniform weight ( $12.5 \pm 0.03$  g) for their size of  $43.2 \text{ cm} \times 27.5 \text{ cm}$ . Measurements were made on alternate days up to 12 d when further accurate measurements were not possible. The results were compared using a *t*-test for the mean % area ( $\pm 1$  s.d.). Animals were housed in groups of four to six during the experiments except in groups *aI* and *bI*. Sialectomy, carried out 7 d before the skin wound, included the submaxillary glands (SMG) and sublingual (SLG) or parotid glands as indicated. Ligation of the submaxillary and sublingual gland ducts was carried out with intervals of 0, 4 or 7 d before wounding.

A  $1\text{-cm}^2$  skin wound was cut into the centre of the back in 110 female C57BL/6J mice aged 50–100 d. Both superficial and deep wounds were studied; the superficial wounds were made by excision of skin only and the deep wounds by excision of skin and panniculus carnosus muscle together. The wound was allowed to dry and retract for 2 h before initial measurement. At 48-h intervals after wounding, the animal was lightly anaesthetised with ether, and the wound area measured. Table 1 summarises the measurements made at 0–12 d in each group. Histological sections were prepared from six controls and seven sialectomised animals on alternate days up to 12 d. Paraffin sections were prepared with both haematoxylin and eosin or Gomori Trichrome.

In normal mice, healing rate of suprapannicular (Table 1*aI*, II) and subpannicular (Table 1*bI*, II) wounds were almost the same, the deeper subpannicular wounds showing a slightly faster rate of contraction during the first 48 h. Wound healing in normal mice varied with the caging method (Fig. 1*a*). When the animals were caged separately their wounds healed slowly, with

the formation of a thick eschar after several days. The mice attempted to lick their own wounds but this was prevented by the wound position in the centre of the back. When the animals were caged in groups of four to six, all the wounds underwent marked contraction within 1–4 d. The initial square wounds became stellate, similar to patterns described in rabbits<sup>13</sup> and guinea pigs<sup>14</sup> (Fig. 2). Communal grooming and wound licking were observed whenever animals were housed together, and therefore all subsequent experiments were carried out with grouped mice.

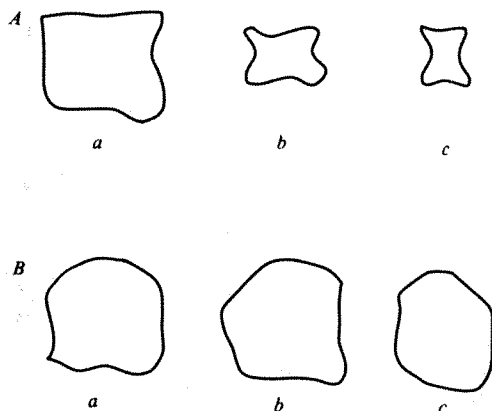
After removal of both the submaxillary and sublingual glands (SMG, SLG), the contraction rate was significantly slower than in sham-operated animals ( $P < 0.001$ ), despite wound licking (Fig. 1*b*). The delayed contraction was similar to that observed in normal animals caged alone, with little change during the first 4 d. The sham-sialectomised mice showed rapid wound contraction similar to non-operated controls ( $P > 0.05$ ).

When sialectomised (SMG, SLG) and sham-operated animals were caged together (Fig. 1*c*), permitting communal wound licking, the healing rates were not significantly different ( $P > 0.05$ ), with all animals showing obvious early wound contraction.

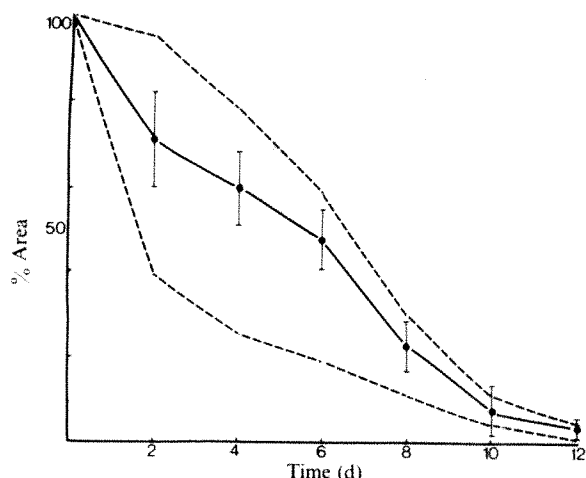
After removal of the submaxillary glands alone, contraction of wounds was slower than in sham-operated controls ( $P < 0.01$ , except day 12). However, contraction was even slower when both submaxillary and sublingual glands were removed ( $P < 0.05$ , except day 10). This result implied that the relatively small sublingual glands, as well as the large submaxillary glands, contained an active substance affecting wound contraction.

Following bilateral parotidectomy the healing rate of wounds approached that observed in sham-operated controls ( $P > 0.05$ , except day 4), with contraction macroscopically indistinguishable from that of controls. This suggested that the parotid glands had no significant role in the effect.

To examine the possibility that the healing effect may be mediated by both exocrine and endocrine routes, experiments with animals which had undergone gland-duct ligation were carried out (Fig. 3). Despite intervals of 0, 4 or 7 d between duct ligation and wounding, healing patterns were not significantly different ( $P > 0.2$ ), and therefore a composite group is shown. Healing was significantly slower than in either non-operated or sham-sialectomised controls ( $P < 0.001$ ), until day 8. When



**Fig. 2** Effect of removal of submaxillary and sublingual glands on early wound shape. *Aa–c* Shows the rapid wound contraction seen in a normal animal at 0, 2 and 4 d, respectively. A wound of a sialectomised animal at 0, 2 and 4 d is shown in *Ba–c*. The wound outlines were traced from photographs, and are approximately  $\times 2$  actual size.



**Fig. 3** Effect of ligation of submaxillary and sublingual ducts on rate of suprapannicular wound healing. Results are expressed as mean % area  $\pm$  1 s.d. for 19 mice (only 12 mice on day 10). *t*-Test showed no statistical difference between the duct-ligated groups despite intervals of 0, 4 or 7 d before wounding, and therefore this figure shows pooled data (●—●). The sialectomised (SMG, SLG) (Table 1aIV) and sham-operated animals (Table 1aIII) are shown as 'ghosts' (---○) for comparison. Wound area was significantly smaller than after sialectomy (SMG, SLG) until day 8 ( $P < 0.05$ ), although it was also larger than in controls until day 8 ( $P < 0.0001$ ).

compared with sialectomised (SMG, SLG) animals, the wounds of the duct-ligated group were significantly smaller up to day 9 ( $P < 0.05$ ), suggesting that saliva was required for rapid wound contraction, and also the possibility of an early endocrine-mediated response. Even after sialectomy, epithelialisation was apparently normal on histological study, but the cause of delayed contraction was not determined.

The rate of wound contraction observed in this study is greater than that reported by other workers<sup>15</sup>, although animals have rarely been permitted or observed to lick their wounds<sup>16</sup>. We considered that the sialectomy wound might itself affect subsequent wound healing, although evidence of different healing rates between primary and secondary wounds is controversial<sup>13</sup>. However, as wounds in non-operated and sham-operated controls contracted equally fast, this possibility can be discounted.

The difference between wounded mice caged individually or in groups was the occurrence of communal wound licking, suggesting an effect either of licking itself or of a salivary gland factor. The mechanical action of the tongue, by minimising scab thickness, could reduce any splinting effect of the scab delaying wound contraction. The scab was certainly thinnest in communally licked controls (or sham-operated animals) with the fastest contraction, although this occurred in the first 2–4 d, before the scab became protuberant. The marginally faster healing in sialectomised animals caged together, compared with controls caged individually, suggested that there was a slight mechanical effect; however, any major effect was unlikely in view of the much slower healing observed in sialectomised animals licked by sialectomised companions than in controls similarly grouped.

Recent evidence suggests that contraction may be controlled by myofibroblasts within granulation tissue<sup>17</sup>. These modified fibroblasts contain smooth muscle-like contractile proteins as well as endoplasmic reticulum for collagen synthesis<sup>18</sup>. Contraction can be experimentally inhibited by smooth-muscle antagonists<sup>19</sup>, but there are no reported methods of its acceleration.

It is uncertain whether sialectomy (SMG, SLG) completely or only partially inhibited contraction; also, the mechanism by which contraction was affected is unknown. Vasoactive substances may accelerate the inflammatory response; kallikrein, which stimulates smooth muscle<sup>20</sup>, may also be involved

in myofibroblast contraction. Alternatively, an unrecognised salivary gland factor may facilitate fibroblast migration, or the differentiation of myofibroblasts, although the origin of these cells is still uncertain<sup>21</sup>.

These results suggest that the mouse submaxillary and sublingual salivary glands contain a factor(s), largely applied by licking, which accelerates early wound contraction. This may be of practical significance in human surgery, as wound contraction is believed to be similar in all mammals. The panniculus carnosus in animals merely allows faster contraction because of greater skin mobility<sup>12</sup>. The next step towards a practical use of our finding would be the isolation of the salivary gland healing factor postulated here.

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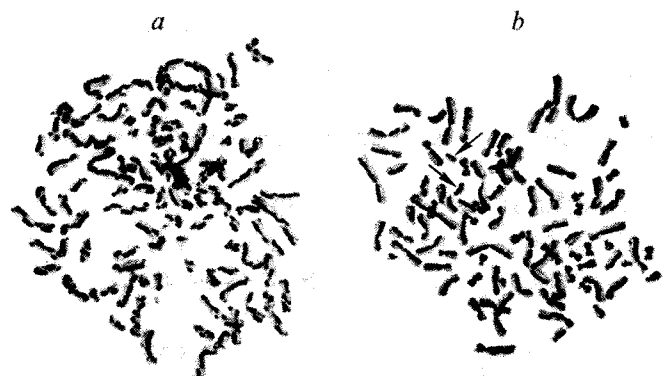
## Normalisation of sister chromatid exchange frequencies in Bloom's syndrome by euploid cell hybridisation

BLOOM'S syndrome (BS) is a rare autosomal recessive genetic disorder characterised by stunted growth, Sun-sensitive telangiectatic erythema of the face and an abnormally high risk of cancer<sup>1</sup>. Cultured lymphocytes and fibroblasts from BS patients have a high frequency of spontaneous structural chromosome aberrations and characteristically high levels of sister chromatid exchanges (SCE)<sup>2</sup>. The primary defect at the molecular level is unknown, but the cytological findings are compatible with a deficiency in a DNA repair function<sup>3</sup>. The presence of a defect of DNA replication is also suggested by the demonstration that the rate of fork motion during replication in BS is slower than normal<sup>4</sup>. German *et al.*<sup>5</sup> have reported the coexistence of cells with both high and normal levels of SCE, suggesting that the defect in BS is regulatory. Tice *et al.*<sup>6</sup> concluded from co-cultivation experiments that SCE frequency is mediated by a diffusible agent(s) present in the medium of BS fibroblasts. Other investigators, however have shown suppression of SCE in conditions of co-cultivation<sup>7,8</sup>. We have clarified the conflict

between these results by examining euploid somatic cell hybrids between BS and normal human fibroblasts.

In the absence of selectable biochemical markers, we obtained hybrids by visual identification of tetraploid clones emerging from fusogen-treated cell mixtures<sup>9,10</sup>. Equal numbers of both parental cell types were co-cultivated for 36 h and then treated with a combination of polyethylene glycol and dimethyl sulphoxide (DMSO)<sup>11</sup>. After 10–12 days presumptive tetraploid colonies were located by phase contrast microscopy<sup>12</sup>, isolated and screened electrophoretically for heteropolymeric glucose-6-phosphate dehydrogenase (G6PD)<sup>13</sup>. Cytogenetically, hybrid clones were tetraploid with two Y chromosomes of different size and heterochromatin content. A reversion to normal levels of SCE in the BS parental phenotype (Fig. 1a and b) was noted in six independent hybrids isolated. Only three of these hybrid clones could be propagated sufficiently for subsequent quantitative analysis of the frequency of SCE. Additional metaphases were classified subjectively as 'low SCE' or 'high SCE', corresponding to the normal and the BS parental SCE phenotype, respectively. Results of these quantitative and qualitative analyses (Table 1) demonstrate that fusions of mutant (high frequency of SCE) with normal (low frequency of SCE) genomes result in complete correction of the mutant phenotype. In contrast, tetraploid clones resulting from fusion between BS parental cells retained the characteristic high frequency of SCE (Table 1).

The possibility that the observed normal low levels of SCE in the hybrids could arise from preferential fusion of the low SCE parent with a subset of BS cells which have low levels of SCE is unlikely for two reasons. First, there was no evidence for bimodality<sup>4</sup> within the BS fibroblast metaphases as regards levels of SCE: the parental BS cells uniformly showed characteristic high levels of SCE (Table 1). Second, in repeated collections, 20–25% of the BS parental metaphases included a small centric fragment derived from an apparently stable rearrangement involving acrocentric chromosomes. These cells were able to form complementing hybrids with normal cells, as observed in hybrid 24, which contained the centric fragment in all metaphases analysed. Thus, at least two karyotypically distinctive subsets of cells from this BS strain had SCE phenotypes which were recessive in hybrids with normal cells. In addition,



**Fig. 1** Cell cultures were grown in Waymouth media supplemented with 16% heat-inactivated FCS. The same lot of serum was used throughout the experiment to control for variation in levels of spontaneous SCE due to serum<sup>15</sup>. The BS strain (GM 1492; Human Genetic Mutant Cell Repository, Camden) was G6PD type B, had a variant size Y chromosome (q12; CBG40)<sup>16</sup> and had chromatid gaps, breaks and rearrangements in more than 16% of its metaphases in three subsequent collections. The G6PD type A strain was derived in our laboratory from neonatal foreskin. For analysis of SCE, cells were exposed to BUdR ( $3 \mu\text{g ml}^{-1}$ ) for 44 h in  $75\text{-cm}^2$  tissue culture flasks. Colcemid ( $0.04 \mu\text{g ml}^{-1}$ ) was added 2 h before collection and slides were prepared according to standard cytogenetic techniques. Differential staining of chromatids was accomplished with heat and Giemsa according to the method of Korenberg and Freedlander<sup>17</sup>. *a*, G6PD B type (BS) tetraploid metaphase, showing high SCE phenotype. *b*, hybrid metaphase with low SCE phenotype. Arrows point to the discordant Y chromosomes.

**Table 1** Quantitative and qualitative evaluation of SCE frequencies in parental, diploid, tetraploid and hybrid cultures

Cultures/Clone	G6PD type	No. of SCE per metaphase*	SCE phenotype	
			Low	High
BS parental				
diploid (GM 1492)	B	$75.4 \pm 8.9$ (22)	(0)	(150)†
Normal parental				
diploid (74–30)	A	$7.0 \pm 1.8$ (25)	(50)	(0)
Hybrids				
No. 6	AB	$12.4 \pm 2.7$ (15)	(17)	(0)
No. 9	AB	$10.4 \pm 2.3$ (17)	(22)	(0)
No. 24	AB	$9.2 \pm 1.8$ (15)	(20)	(0)
BS tetraploids‡				
No. 15	B	$129.2 \pm 9.5$ (17)	(0)	(25)
No. 28	B	$133.1 \pm 11.4$ (16)	(0)	(20)
Normal tetraploids				
No. 58	A	$10.5 \pm 1.5$ (6)	(19)	(0)

AB represents the G6PD heteropolymeric enzyme.

\*Values are means  $\pm$  s.d.; numbers of metaphases are shown in parentheses.

†50 Metaphases from each of three independent collections.

‡Isolated concomitantly with hybrid tetraploid clones after fusion treatment and dilution plating.

none of the hybrid clones seemed to display the characteristically high levels of unstable chromatid aberrations of BS cells in first division metaphases (defined by the staining response following incorporation of bromodeoxyuridine (BUdR)). The latter observations require quantification in a larger series of hybrids.

Our results do not support the evidence of Tice *et al.*<sup>6</sup>, which suggested that an endogenous production of a DNA-damaging agent caused the high frequency of SCE in BS cells. Rather, they are consistent with the idea that the high frequency of SCE reflects an intrinsic genetic defect in BS cells themselves.

Metaphases from BS heterozygotes show normal levels of SCE<sup>2</sup>. We plan to determine the level of SCE in hybrids between BS heterozygotes and related BS homozygotes. Such hybrids would contain three abnormal but only a single normal allele for the as yet unidentified gene defect. If such hybrids display intermediate levels of SCE, they would serve as a useful test system for heterozygosity<sup>14</sup>. Our technique of euploid–euploid hybridisation might, in addition, be used to investigate the question of genetic heterogeneity among BS patients<sup>1</sup>.

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## Human brain tumour cell strains with deficient host-cell reactivation of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine-damaged adenovirus 5

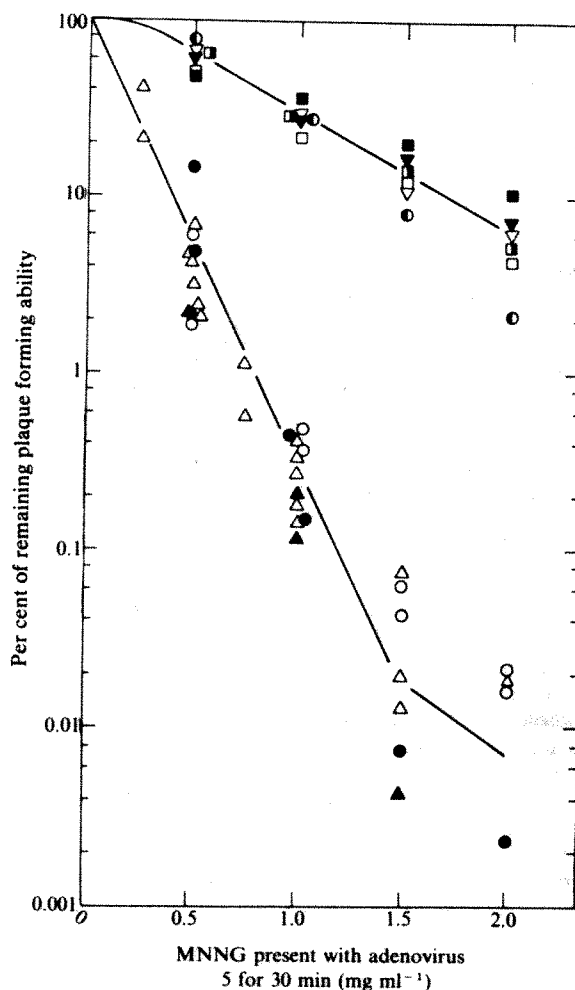
HOST-CELL reactivation of a population of physically or chemically damaged viruses is said to occur when the plaque titre of the damaged (but not the control) virus population depends strongly on the cell strain selected to serve as the host to support virus growth<sup>1,2</sup>. Such cell strain-dependent differences in survival have classically been interpreted as reflecting known cellular differences in ability to repair DNA<sup>1,2</sup>. For example, we have previously demonstrated host-cell reactivation of UV-irradiated<sup>3</sup> or benzo(a)pyrene diol-epoxide I (anti)-treated<sup>4</sup> human adenoviruses. The survival of such treated virus populations is much greater when their plaque titre is measured using monolayers of fibroblasts with normal DNA repair than when using fibroblasts from patients having the genetic disease, xeroderma pigmentosum, which are deficient in repair of UV-damaged DNA. The work of Lytle and coworkers<sup>5,6</sup> and Day<sup>3</sup>, using human cells, has shown host-cell reactivation of UV-damaged viruses to occur only when nuclear replicating DNA viruses are studied. To assess the hypothesis that human tumorigenesis is often associated with repair-deficient cells, we undertook a study of host-cell reactivation of various kinds of damage using human cell strains prepared both from tumours of different organs and from skin of people having genetic predisposition to, or familial occurrence of, cancer. As part of this work, we measured the survival of adenovirus 5 treated *in vitro* with the carcinogen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG). We found that the use of cells from 4 of 13 human brain tumour strains as viral hosts resulted in less survival of the MNNG-damaged viruses than did the use of more than 20 other cell strains prepared from human tumours or from unaffected human organs (Fig. 1, Table 1). Previous studies have not detected abnormal DNA repair in human tumours<sup>7</sup>. We believe this to be the first report of host-cell reactivation of MNNG damage by mammalian cells.

Data were obtained in eight experiments with A172 (Fig. 1, data from six) and in two each with A382, U-87MG and U-105MG. A172 was established from a glioblastoma<sup>8</sup> (a glioblastoma is a highly malignant (Grade IV) astrocytoma), the other three from astrocytomas<sup>8-10</sup>. Six other cell strains (T-98, 118MG, U-373MG, U-251MG, U-178MG and U-138MG) also established from glioblastomas or astrocytomas<sup>9,10</sup> showed approximately the same level of host-cell reactivation as the 23 remaining strains listed in Table 1 (data obtained using three of the six are included in Fig. 1). Among the strains showing normal reactivation of MNNG-damaged adenovirus 5 were three skin fibroblast strains prepared from patients having ataxia telangiectasia—AT3BI, AT5BI and AT81CTO (inactivation data for the first two of these is given in ref. 11). The colony forming ability of strain AT3BI has been found to be more sensitive to inactivation by MNNG than that of the KD, CRL1187, and CRL1220 normal strains<sup>12</sup>. One cell strain from a xeroderma pigmentosum patient, XP12BE, previously shown to be highly defective in host-cell reactivation of both UV-damaged<sup>3</sup> and benzo(a)pyrene diol-epoxide I (anti)-damaged<sup>4</sup> adenovirus 5, shows a normal level of host-cell reactivation of MNNG-damaged virus. Three skin fibroblast cell strains from persons with brain tumours, and belonging to cancer-prone families<sup>13-15</sup>, also show normal reactivation of the MNNG-damaged virus.

Although A172, U-87MG and U-105MG host cells reactivate MNNG-damaged adenovirus 5 poorly, Fig. 2 shows that they have normal host-cell reactivation of UV-irradiated viruses. The UV sensitivity of the virus reflected by the slope of the inactivation curve is experimentally indistinguishable from

that previously reported using 10 normal human skin fibroblast strains<sup>3</sup>. Thus the defective host-cell reactivation of MNNG-damaged adenovirus 5, characteristic of these strains, does not reflect a general decrease in their ability to support the growth of damaged adenoviruses. (While A172, U-87MG and U-105MG grow very well, the slow growth of A382 has slowed further experimentation, and UV survival was not tested with this strain.)

We interpret our results to mean that the A172, A382, U-87MG and U-105MG cell strains are defective in the ability to repair MNNG-damaged adenovirus 5. While we believe the MNNG lesion, which is not repaired well by these strains, to be



**Fig. 1** Per cent remaining plaque forming ability of adenovirus 5 as a function of MNNG concentration present with the virus. Human adenovirus 5, purified as previously described<sup>4</sup>, was diluted 1:1000 in 0.3 M Tris, pH 9.0. Aliquots (0.1 ml) of ethanol stock solutions of appropriate concentrations of MNNG (Aldrich) were added to 0.9-ml portions of the diluted virus suspension. After 30 min incubation at 37 °C, 0.1 ml of 0.5 M *N*-acetyl-L-cysteine<sup>16</sup>, pH 7.2, was added to each treated virus sample to reduce the remaining MNNG and thereby to terminate the viral inactivation. After 5–15 min further incubation, samples were diluted 1:10 into basal medium Eagle's medium (BMEM) (Microbiological Associates) containing 1% fetal bovine serum (GIBCO) plus 50 U penicillin and 50 µg streptomycin per ml. Plaque assays were then carried out as described previously<sup>3,4,20</sup>. The strains used, followed by the respective number of adenovirus 5 plaque forming units per ml from the dilution of non-treated viruses into BMEM were: ▲, A382,  $11.4 \times 10^5$ ; ●, U-87MG,  $30.2 \times 10^4$ ; ■, U-373MG,  $50.8 \times 10^4$ ; ○, U-105MG,  $45.4 \times 10^5$ ; □, U-178MG,  $53 \times 10^4$ ; △, A172,  $36.3 \times 10^4$ ; ▼, H4,  $90.8 \times 10^4$ ; ▽, 118MG,  $92.4 \times 10^3$ ; ○, CRL 1187,  $16.2 \times 10^4$ ; and ■, HEK,  $13.5 \times 10^5$ . Several points are displaced for clarity. Further cell strain data are given in Table 1 legend.

Table 1 Cell strains used

Human tumour cell strains	Tumour of origin	Donor age, sex	Skin fibroblast cell strains	Characterisation	Donor age, sex
Reactivation deficient			Reactivation proficient		
A172	Glioblastoma (astrocytoma, grade IV)	53, M	CRL 1220	Apparently normal	15, M
A382	Astrocytoma	10, M	CRL 1187	Apparently normal	14, M
U-87MG	Astrocytoma, grade II	54, F	CRL 1224	Apparently normal	40, F
U-105MG	Astrocytoma, grade III	62, M	KD (CRL 1295)	From blood blister of lip	31, F
			WR001	Cockayne's syndrome	9, F
			XP12BE (CRL1223)	Xeroderma pigmentosum	7, F
			AT3BI	Ataxia telangiectasia	4, M
			AT5BI	Ataxia telangiectasia	?, M
			AT81CTO	Ataxia telangiectasia	<1, M
118MG	Glioblastoma vs. astrocytoma	50, M	AL1405	Acute myelogenous leukaemia	6, M
U-373-MG	Astrocytoma, grade III	61, M	AL639	Acute monomyelogenous leukaemia	—
U-251MG	Astrocytoma, grade I	75, M	AL377	Brain tumour prone family	19, M
U-178MG	Astrocytoma, grade II	56, M	AL2673	Brain tumour prone family	38, M
U-138MG	Glioblastoma	47, M	AL1899	Brain tumour prone family	35, M
T-98	Glioblastoma	61, M			
Hs783T	Neuroblastoma	4, M			
Hs683	Glioma	76, M			
H4	Neuroglioma	37, M			
A498	Kidney carcinoma	52, F			
A704	Kidney carcinoma	78, M			
Hs775T	Wilms tumour (kidney)	13, M			
Hs703	Liver carcinoma	55, M			
A204	Rhabdomyosarcoma	1, F			
			Other cell strains		
			Reactivation proficient		
			HEK	Human embryonic kidney	—
				Per cent of remaining plaque forming ability	

Cell strains A172, A204, A382, A498 and A704<sup>8</sup>, 118MG, H4, Hs783T, Hs683, Hs703 and Hs775T were from Dr Walter Nelson-Rees and Jack Weaver, with support from the NCI/Viral Oncology program, under the auspices of the Office of Naval Research and the Regents of the University of California. Cell strains T-98, U-87MG, U-105MG, U-138MG, U-178MG, U-251MG and U-373MG were from Dr Jorgen Fogh, Sloan-Kettering. Strains designated by 'CRL' were from the American Type Culture Collection, Rockville. HEK cells were from GIBCO. Strains designated AL were supplied by Dr Anthony Lubiniecki, Meloy (AL377 is from case IV-7 in NIH Clinical Epidemiology Branch family 0068 (ref. 13), AL1899 is from case 3 in family 0272 (ref. 14), AL2673 is from case V-8 in family 0165 (ref. 15), and AL1405 from family 0827). AT3BI and AT5BI were from Dr Colin Arlett and AT81C10 was from Dr M. C. Paterson, Atomic Energy of Canada. Strain WR001 was the gift of Dr Peter Nissley, NCI, NIH. Strain XP12BE belongs to xeroderma pigmentosum complementation group A (ref. 21).

in the DNA of a damaged adenovirion, the identification of this lesion as being an MNNG altered viral protein remains a possibility, although there are no reports of successful cellular repair of damaged proteins.

Candidates for repairable lesions produced by MNNG in adenovirus DNA are methylated bases<sup>16</sup>, phosphotriesters<sup>17</sup> and *N*-nitrocyanamide reaction products<sup>18</sup>. In our experiments, we activated MNNG by low amounts (*pH* 9) of hydroxyl ion, a reaction which gives methyl diazonium hydroxide (which subsequently becomes a methylating agent) and *N*-nitrocyanamide anion<sup>16</sup>. We have found that another type of virus treatment (treatment of virus suspensions by reduction of MNNG using *N*-acetyl-L-cysteine<sup>16</sup>) which produces a strong methylating agent, but does not produce active *N*-nitrocyanamide anion<sup>16</sup>, does not result in viral inactivation. This, by elimination, suggests that the repairable lesion may be a DNA-*N*-nitrocyanamide reaction product. Further results suggesting the possible importance of the *N*-nitrocyanamide moiety come from comparative studies of the kinetics of viral inactivation using MNNG and its ethyl counterpart, ENNG. Although these two agents produce different amounts of alkylated bases and phosphotriesters relative to their total reaction<sup>17</sup> (MNNG gives more total reaction than ENNG), they give the same viral inactivation kinetics per mol using repair proficient cells (data not shown), suggesting that the viral inactivating lesion is due either to the *N*-nitrocyanamide group, common to the two agents, or to some alkylated base or phosphotriester made in quantities such that the biological effects of the two agents are equal.

The fact that the decreased survival of MNNG-treated virus is observed in four of ten cell strains prepared from human astrocytomas suggests four possibilities for the relationship of MNNG host-cell reactivation deficiency to the origin of the astrocytomas that gave rise to the A172, A382, U-87MG and U-105MG cell strains: (1) tumorigenesis leading to astrocytomas is often initiated in a group of cells which is normally

deficient in host-cell reactivation of MNNG-damaged adenovirus; (2) such tumorigenesis often generates host-cell reactivation deficient cells; (3) such tumorigenesis is initiated in a cell, which, by somatic mutation, has become host-cell reactivation deficient; and (4) a large proportion of astrocytomas

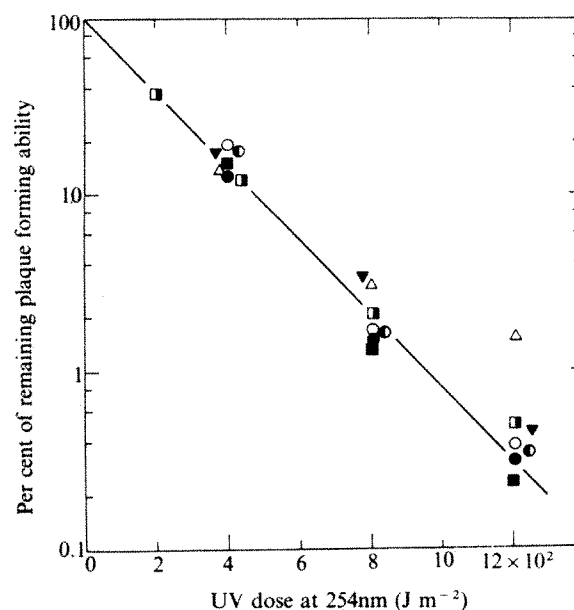


Fig. 2 Per cent remaining plaque forming ability of adenovirus 5 as a function of UV dose to the virus. Purified human adenovirus 5 was diluted 1:1,000 in PBS, UV-irradiated, and assayed for plaque forming ability<sup>3,4,20</sup>. The strains used, followed by the number of adenovirus 5 plaque forming units per ml PBS were: ●, U-87MG,  $18.0 \times 10^5$ ; ○, U-105MG,  $30.4 \times 10^5$ ; △, A172,  $84.7 \times 10^4$ ; ▼, H4,  $22.6 \times 10^6$ ; □, CRL 1187,  $29.9 \times 10^4$ ; and ■, HEK,  $21.6 \times 10^6$ . See Table 1 legend for further strain data.

arise in people who have a genetic defect manifested by impaired host-cell reactivation of MNNG-treated adenoviruses. If the last possibility were correct, the disease identified would be another of the genetic diseases in which defective cellular repair of damaged DNA is associated with carcinogenesis. The three diseases known to be of this type, xeroderma pigmentosum, ataxia telangiectasia and Fanconi's anaemia, are infrequently associated with brain tumours<sup>19</sup>.

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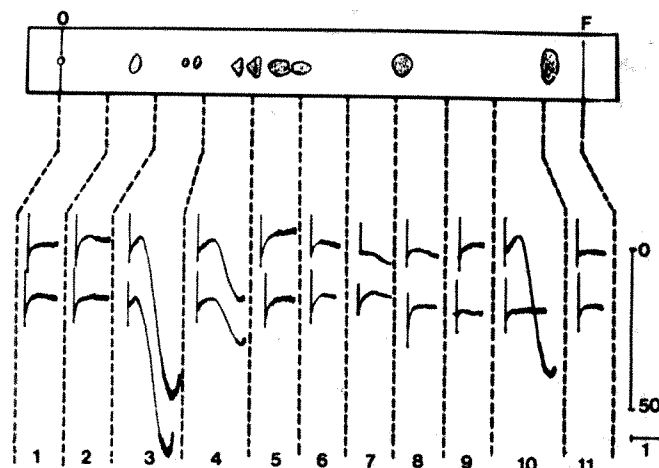
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## The role of platelet-activating factor in platelet aggregation

PLATELET aggregation is mediated by at least three distinct mechanisms<sup>1,2</sup>. The first involves the release of ADP and is inhibited by its conversion to ATP by the combination of creatine phosphate and creatine phosphokinase (CP/CPK). The second is mediated by metabolites of arachidonic acid, particularly thromboxane A<sub>2</sub> (TXA<sub>2</sub>), and is blocked by aspirin or indomethacin, inhibitors of the arachidonate cyclo-oxygenase pathway<sup>3</sup>. It has been postulated that a third mechanism must exist, as neither CP/CPK nor aspirin, alone or combined, can inhibit aggregation induced by high concentrations of thrombin or the calcium ionophore A23187 (refs 1, 2, 4). Antigenic challenge of IgE-sensitized basophils releases a platelet-activating factor (PAF), probably a 1-lysophosphatidylcholine<sup>5</sup>. PAF has a potent action on rabbit<sup>6,7</sup> and human<sup>8,9</sup> platelet aggregation and release which is independent of the cyclo-oxygenase arachidonate pathways<sup>6,10,11</sup>. We have also obtained PAF from A23187-stimulated rat peritoneal<sup>12</sup> and alveolar (J.B., B.



**Fig. 1** Separation by TLC of aggregating substances released during ionophore-induced platelet aggregation. The upper part of the figure shows the thin layer chromatogram obtained by running a 10-μl aliquot of the 100 μl of the chloroformic platelet lipid extract. Spots were revealed with iodine vapour. The 90 μl remaining was run in parallel and divided into 11 square zones. Aliquots of 1 μl of the eluate from each zone were added to 0.35 ml of rabbit washed platelets ( $5 \times 10^8$  platelets per ml) incubated for 15 s with (lower tracings) or without (upper tracings) 5 μM indomethacin. Two distinct areas were detected: eluates from zones 3 and 4 (left hand) induced aggregation which was unaffected by indomethacin and can thus be considered as cyclo-oxygenase independent. Eluate from zone 10 (right hand) induced aggregation which was completely blocked by indomethacin, indicating the presence of arachidonic acid. Vertical scale: % light transmission; horizontal scale: time in minutes.

Arnoux and D. Duval, in preparation) macrophages. Moreover, thrombin and ionophore-induced platelet aggregation and the accompanying stimulation of phospholipase A<sub>2</sub> (refs 13, 14) are inhibited by the phospholipase inhibitor bromophenacyl bromide (ref. 15 and B.B.V., F. Fouque and M.C., in preparation), suggesting that the third mechanism of platelet aggregation might involve a lipid mediator. These findings prompted us to investigate whether platelets can form and release PAF in experimental conditions in which the ADP and TXA<sub>2</sub> pathways are fully blocked. We report here that this is indeed the case, and suggest PAF as a likely candidate for mediating the 'third pathway' of platelet aggregation.

In 12 experiments, blood was collected in EDTA (2.5 mM) from the ear artery of rabbits, and platelet-rich plasma obtained by standard centrifugation procedures. Washed platelets were prepared according to the method of Ardlie *et al.*<sup>16</sup> modified as described elsewhere<sup>6</sup>. In two experiments human blood was collected on citric acid–citrate–dextrose (for 9 ml of blood, 1 ml of a solution made of 8 g citric acid monohydrate, 22 g citrate trisodium dihydrate and 22.3 g glucose per l of distilled water); platelets were washed by two centrifugations at 1,000g for 15 min on a cushion of erythrocytes and then separated by centrifugation at 90g for 15 min (modified by J.B. from ref. 17). Washed platelets from rabbits ( $5 \times 10^9$ ) and from humans ( $3 \times 10^9$ ) were suspended in 10 ml of Tyrode's solution containing 2.5 g l<sup>-1</sup> bovine serum albumin (fraction V, Armour) and buffered with Tris (Sigma), 0.01 M, at pH 7.4. Platelets were stirred at 37 °C with or without 2.5 μM A23187 (Lilly). In four experiments aspirin (Prolabo) and the CP/CPK enzymatic system were added to platelets 10 min before the ionophore. In every experiment, the concentrations used, 0.1 mM for aspirin and 31.25 μg ml<sup>-1</sup> and 15.25 μg ml<sup>-1</sup> for CP and CPK (Sigma), respectively, were able to block aggregation induced by 0.1 mM arachidonic acid and to convert 50 μM ADP after 1 min incubation.

Ten minutes after the addition of the ionophore the suspension was ultrasonicated for 30 s in a Branson ultrasonicator, and the pH was adjusted to 10.6 with NaOH. Incubates were stirred



**Table 1** Release of platelet-activating factor and arachidonic acid from rabbit and human platelets during A23187-induced aggregation

		Additions		
		None	A23187	A23187 Aspirin and CP/CPK
Rabbit	PAF	0	1,957 ± 1,147	1,797 ± 740
	AA	756 ± 576	1,197 ± 653	732 ± 282
Human	PAF	0; 0	625; 725	ND
	AA	0; 0	145; 130	ND

Washed platelets were incubated for 10 min with or without A23187, a combination of aspirin and CP/CPK having been added 10 min before the ionophore (see text). Numbers represent platelet-aggregating activities expressed in arbitrary units per ml of incubate, recovered after TLC. PAF, activities from zone 3, indomethacin resistant; arachidonic acid (AA), activities from zone 10, indomethacin sensitive. Results of rabbit experiments are the mean ± s.d. of 12 experiments on  $5 \times 10^8$  platelets per ml; those of humans are from two separate experiments using  $3 \times 10^8$  platelets per ml. Note that arachidonic acid was also released from platelets used as controls, probably due to damage during extraction. ND, not done.

for 1 h at room temperature and 1.5 volumes of absolute ethanol were then added. The mixture was evaporated overnight under a stream of air and the residue was extracted twice with 1 ml of chloroform. After evaporation the new residue was dissolved in 100 µl of chloroform. Samples were spotted on silica-gel TLC plates (Merck, 60F-254) which were developed with a chloroform/methanol/water mixture (70:35:7). The plates were divided into 11 squares of  $1.5 \times 1.5$  cm, and the gel from each square was scraped and eluted with 500 µl of 60% ethanol. Eluates, usually 1-µl aliquots, were assessed for their aggregating activity on rabbit washed platelets as described elsewhere<sup>6</sup>.

Platelet aggregation was induced by eluates from two main areas (Fig. 1): zones 3 and 4, which had an  $R_F$  of ~0.35, similar to that of hog leukocyte PAF<sup>12</sup>, and zone 10, with the  $R_F$  of standard arachidonic acid<sup>12</sup>. The aggregating activity recovered from zones 3 and 4 was unaffected when 5 µM indomethacin was added to platelets before challenge (Fig. 1). In contrast, the eluates from zone 10 were totally inactive in the presence of the cyclo-oxygenase inhibitor, indicating that the aggregation was due to arachidonic acid (Fig. 1). Activities of arachidonate and PAF were expressed in arbitrary units—1 unit induced an aggregation producing a 1% change in light transmission.

In these experiments, PAF activity with a range of 565–4,000 U ml<sup>-1</sup> was obtained. In five control experiments no PAF was found when platelets were processed without the addition of the aggregating agent (Table 1). Preincubation of platelets for 10 min with aspirin and CP/CPK before addition of the ionophore did not affect aggregation or the formation and release of PAF. Human platelets also formed PAF on stimulation with the ionophore, although in lower amounts, even considering the lower number of human platelets in the release experiments. The presence of Ca<sup>2+</sup> was an absolute requirement for the release of PAF, as 2 mM EDTA suppressed it completely.

PAF purified on TLC was totally inactivated by porcine pancreatic phospholipase A<sub>2</sub> (Boehringer)<sup>5</sup>, an enzyme which specifically hydrolyses the fatty acid in the 2 position of glycerophospholipids. Furthermore, in two experiments TLC-purified PAF from rabbit platelets exhibited a similar retention time to that of PAF from hog leukocytes, when re-chromatographed using high-pressure liquid chromatography (HPLC). Therefore, PAF obtained from rabbit platelets fulfilled the criteria necessary to differentiate it from other potential aggregating agents<sup>5,12</sup>: refractoriness to indomethacin and to CP/CPK, sensitivity to phospholipase A<sub>2</sub>, solubility in organic solvents and typical migration pattern on silicic acid TLC and HPLC. PAF from human platelets was also insensitive to indomethacin and CP/CPK, was fully destroyed by phospho-

lipase A<sub>2</sub> and exhibited the same migration pattern as PAF from rabbit platelets or hog leukocytes on TLC (not tested on HPLC).

In seven experiments, rabbit platelets were centrifuged for 10 min after ionophore-induced aggregation. Sonication and exposure to basic pH were omitted. The recovery of  $63 \pm 29\%$  of total PAF activity in the supernatants indicated that PAF was released by aggregating platelets into the extracellular medium. The overall release of PAF by the ionophore-stimulated platelets was very high. Indeed, the amount of PAF released from rabbit platelets was 10–50 times greater than that required to aggregate 100% of a physiological platelet concentration mimicked in an aggregometer tube. PAF released from platelets may thus induce aggregation and secretion of the majority of platelets in the immediate vicinity, a basic mechanism for the formation of the platelet plug during haemostasis.

Our data indicate the existence of a calcium-dependent platelet mechanism leading to the formation and release of PAF during aggregation. This release occurs equally well whether the ADP and TXA<sub>2</sub>-dependent mechanisms are activated or whether they are inhibited by a combination of aspirin and CP/CPK. This strongly suggests that PAF is the mediator of the putative third pathway of platelet aggregation.

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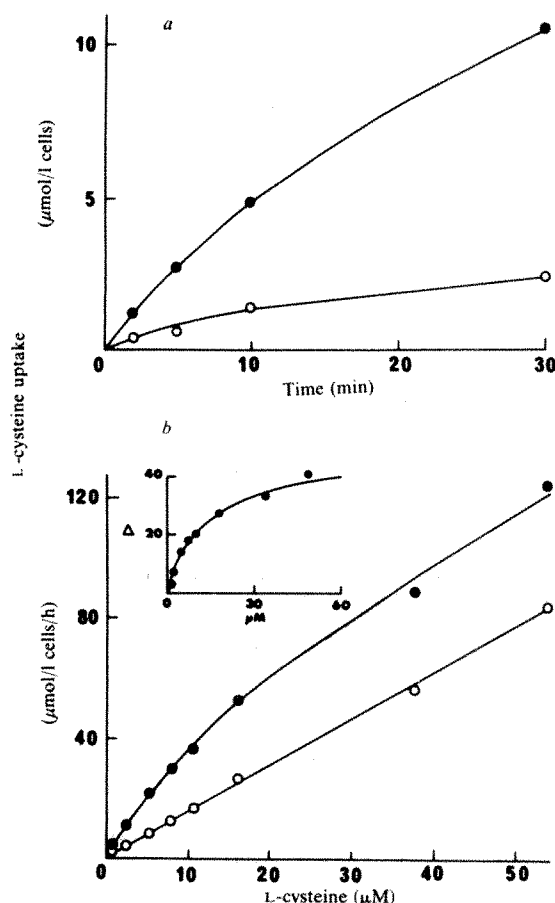
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## Sodium-dependent cysteine transport in human red blood cells

ADEQUATE membrane transport of L-cysteine is necessary for red blood cell (RBC) survival, as this amino acid is essential for glutathione (GSH) synthesis and thus cell protection against oxidative damage. Thus, in sheep a genetically controlled L-cysteine transport defect leads to a reduced intracellular GSH concentration and shortened cell lifespan<sup>1–4</sup>. Human RBCs do not have the same amino acid transport system as sheep red blood cells (SRBC)<sup>5</sup>, the principal component being a high capacity, medium affinity system with a specificity broadly directed towards large neutral amino acids, the L-system of Christensen<sup>6</sup>. L-cysteine and its analogue L-α-amino-n-butyrate are carried by the L-system in human RBCs, but with a low affinity<sup>7</sup>. Most experiments on amino acid transport in RBCs,



**Fig. 1** *a*, Time course of L-cysteine uptake by human RBCs in sodium (●) and choline medium (○). *b*, Concentration dependence of L-cysteine uptake by human RBCs in the presence (●) and absence of sodium (○). Freshly drawn human RBCs were washed three times in medium containing 150 mM NaCl or choline Cl, 15 mM Tris-HCl (pH 7.6 at 37°C) and 5 mM glucose. The buffy coat was discarded. L-cysteine uptake was measured at 37°C by mixing 0.15 ml prewarmed cell suspension (haematocrit approximately 20%) in the appropriate medium with 0.15 ml prewarmed Na or choline medium containing 20 mM dithiothreitol and 1–50 μM L-cysteine (including L-U-<sup>14</sup>C-cysteine (0.5 μCi μmol<sup>-1</sup>); Radiochemical Centre, Amersham, UK). Incubations were stopped at predetermined time intervals by the addition of 1 ml ice-cold magnesium chloride solution (107 mM Mg Cl<sub>2</sub>, 10 mM Tris-HCl, pH 7.6 at 4°C). Cells were rapidly washed four times in this medium using an Eppendorf microcentrifuge (10s, 15,000g). Finally the washed cell pellets were lysed with 0.5 ml 0.5% (v/v) Triton X-100 in water, and 0.5 ml 5% (w/v) trichloroacetic acid was added. The precipitate was removed by centrifugation, and radioactivity in the protein-free supernatants determined by β-scintillation counting with quench correction. The time course studies (*a*) were made at a final L-cysteine concentration of 10 μM. The insert curve in (*b*) is fitted as  $\Delta = 49.83S/(14.2 + S)$  where  $\Delta$  is the difference between Na and choline uptake rates (μmol per l cells per h) and  $S$  is the L-cysteine concentration (μM). All values are means of duplicate estimates.

particularly involving the L-system, have revealed substrate affinities with apparent  $K_m$  values in the millimolar range, much higher than the physiological plasma levels of most amino acids. We have now investigated L-cysteine uptake in human RBCs over the concentration range 1–50 μM, as human plasma levels are around 20 μM<sup>8,9</sup>. As the early work of Yunis and Arimura<sup>10</sup> has shown that glycine and L-alanine transport are partially Na-dependent, we have also investigated the effects of removing external Na on L-cysteine transport. We report here that human RBCs transport L-cysteine by a previously unidentified high affinity, low capacity, Na-dependent uptake mechanism. This system has a uniquely high affinity for its substrate and is the first Na-dependent transport mechanism to be kinetically characterised in mammalian erythrocytes. At physiological substrate

concentrations it accounts for approximately half of the L-cysteine uptake into the cell.

Figure 1*a* shows the time course of L-cysteine uptake (extracellular concentration 10 μM) in either Na or choline medium. Uptake in both cases was linear over the first 10 min of incubation, with the flux in choline medium threefold lower than in the presence of Na. Replacement of Na by other cations gave inhibitions of 61% choline replaced; 65% Mg replaced; 54% Li replaced and 41% K replaced, suggesting that the reduced uptake was a true Na-replacement response. Extended incubation of cells (2 h) in the presence of 10 μM L-cysteine resulted in label accumulating to 33.4 and 15.4 μmol/l cell water in Na and choline medium, respectively. Figure 1*b* presents the concentration dependence curve for the initial rate of L-cysteine uptake in either Na or choline medium. The flux in choline medium was linear, whereas the Na medium data were consistent with the addition of a saturable component obeying simple Michaelis-Menten kinetics with apparent  $K_m$  and  $V_{max}$  values of 14.2 μM and 49.8 μmol/l cells/h, respectively. Kinetic studies on three individuals gave apparent  $K_m$  and  $V_{max}$  values (mean ± s.e. (range)) of  $17.6 \pm 1.7$  (14.2–20.0) μM and  $53.9 \pm 10.2$  (38.5–73.3) μmol/l cells/h, respectively.

The possibility that white cell or platelet contamination of the RBC population was responsible for the Na-dependent L-cysteine uptake was eliminated by comparing Na-dependence before and after filtration to remove white cells and platelets<sup>11</sup>, when fluxes were unaffected. The presence of reticulocytes was monitored by brilliant cresyl blue staining<sup>12</sup>. Reticulocyte counts were in the range 0–1.4% for the three subjects used, and there was no correlation between fluxes and reticulocyte counts. The magnitude of L-cysteine uptake over extended time periods is an additional argument against this effect resulting from L-cysteine accumulation in a minor component of the total cell population. The fact that the label accumulated to a level three times greater inside the cells suggests a possible concentrative effect. However, intracellular L-cysteine metabolism may be considerable, and makes it impossible to directly relate isotope accumulation to free intracellular L-cysteine levels. Further experiments using L-alanine<sup>14</sup>, an amino acid which is not significantly metabolised in RBC, revealed a Na-dependent uptake, confirming the observations of Yunis and Arimura<sup>10</sup>, and L-alanine was found to be an effective inhibitor of Na-dependent L-cysteine uptake. Therefore, as these two amino acids seem to share the transport system, L-alanine may prove a more convenient substrate for characterising this system in more detail. Further experiments measuring Na-dependent alanine uptake in human RBCs separated according to density on a Percoll gradient<sup>15</sup> gave 56% Na-sensitive alanine uptake (alanine concentration 0.2 mM) in the young cell fraction, and 43% in the densest fraction, corresponding to the old cells.

Increasing intracellular Na using the nystatin method<sup>16</sup> from 14 to 95 mmol/l cell water, decreased the Na-dependent uptake by 50% (external Na 150 mM). Neither ouabain 1 mM nor furosemide 1 mM inhibited Na-dependent amino acid uptake. At an alanine concentration of 0.5 or 1 mM, it was also possible to demonstrate an amino acid-dependent Na uptake, the mean Na/alanine coupling ratio being  $1.15 \pm 0.06$  (ref. 4).

The present results suggest that normal human RBCs possess a high affinity, low capacity Na-dependent L-cysteine transport system. The kinetic parameters of this system relate well to free L-cysteine plasma levels<sup>8,9</sup>, and intracellular L-cysteine requirements for GSH biosynthesis derived from consideration of intracellular GSH turnover<sup>13</sup>. We therefore conclude that this transport system may represent a major pathway for L-cysteine entry into human RBCs *in vivo*.

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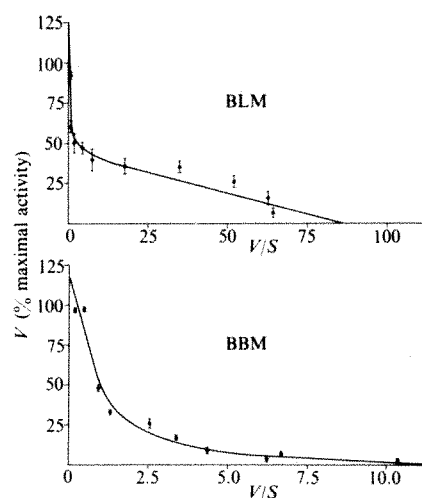
## Ca-stimulated ATPase in brush border and basolateral membranes of rat duodenum with high affinity sites for Ca ions

THE small intestine plays an important part in the calcium homeostasis of the body, but the mechanism by which calcium is absorbed is poorly understood. In young rats, active calcium transport occurs primarily in the duodenum and is strongly dependent on the vitamin D status of the animal<sup>1,2</sup>. In brush border membranes (BBM) as well as in basolateral membranes (BLM) the presence of Ca<sup>2+</sup>-ATPase activity has been reported<sup>3–5</sup>. Moreover, a good correlation is found between Ca<sup>2+</sup>-ATPase activity and net calcium transport in different segments of rat small intestine<sup>6</sup>. These observations suggest a role for Ca<sup>2+</sup>-ATPase in calcium absorption. The electrochemical potential of Ca<sup>2+</sup> in intestinal cells suggests that Ca<sup>2+</sup> influx is passive, whereas extrusion of Ca<sup>2+</sup> must be active<sup>6</sup>. Inherent in a role for Ca<sup>2+</sup>-ATPase in calcium transport is the requirement that Ca<sup>2+</sup>-ATPase be stimulated by concentrations occurring in the cytosol ( $\leq 10^{-5}$  M). So far, Ca<sup>2+</sup>-ATPase activities in small intestine have been assayed in the presence of 2 to 40 mM Ca<sup>2+</sup> (refs 3–5). We report here that Ca<sup>2+</sup>-ATPases in BBM and BLM of rat duodenum have  $K_m$  values for Ca<sup>2+</sup> activation of 1.1 and 0.5  $\mu$ M respectively. Also Ca<sup>2+</sup>-ATPase activity below 5  $\mu$ M Ca<sup>2+</sup> is higher in BLM than in BBM fragments. This asymmetrical distribution of Ca<sup>2+</sup>-ATPase activity may provide a mechanism for net calcium transport from lumen to blood.

**Table 1** Ca<sup>2+</sup>-ATPase activities of BBM and BLM fragments at various Ca<sup>2+</sup> concentrations

[Ca <sup>2+</sup> ] ( $\mu$ M)	Specific activity/purification factor ( $\mu$ M Pi per h per mg protein)	
	BBM	BLM
0.5	0.043 $\pm$ 0.010	0.12 $\pm$ 0.02
1.0	0.091 $\pm$ 0.023	0.17 $\pm$ 0.01
5.0	0.23 $\pm$ 0.02	0.18 $\pm$ 0.04
100.0	1.28 $\pm$ 0.03	0.42 $\pm$ 0.13

Observed specific activities (mean  $\pm$  s.e.m.,  $n = 5$ ) were divided by the purification factors of the BBM or BLM respectively. The purification factor for (Na<sup>+</sup>K<sup>+</sup>)ATPase, a BLM marker is  $7.9 \pm 1.1$  ( $n = 5$ ), while for sucrase, a BBM marker is  $39.8 \pm 2.7$  ( $n = 5$ ). These purifications are comparable to previously published studies<sup>5,6</sup>.



**Fig. 1** Eadie-Hofstee plots of Ca<sup>2+</sup>-ATPase activity at various calcium concentrations. Enzymatic activity is expressed as percentage of maximal Ca<sup>2+</sup>-ATPase activity, for example 3.6 and 54.4  $\mu$ M Pi per h per mg protein for BLM and BBM respectively. Calcium concentrations are expressed in  $\mu$ M. The data have been fitted based on a general rate equation for a two-site transport model<sup>17</sup> by means of an iterative procedure (least-squares method). Extrapolated  $V_{max}$  values for the high affinity sites are 1.58 and 6.53  $\mu$ M Pi per h per mg protein in BLM and BBM respectively. These  $V_{max}$  values represent 45% and 12% of the total Ca<sup>2+</sup>-ATPase activity at 1 mM Ca in BLM and BBM fragments.  $K_m$  values for the high affinity sites are 0.5 and 1.1  $\mu$ M in BLM and BBM.  $K_m$  values for the low affinity sites are 50 and 70  $\mu$ M for BLM and BBM respectively.

BLM and BBM were isolated from the first 15 cm of the small intestine of male Wistar rats (140–160 g) using the method described by Mircheff and Wright<sup>5,7</sup>. Plasma membrane fractions were essentially free of mitochondria, as succinic dehydrogenase (SDH) activity was  $\leq 0.2\%$  of the initial activity. This is important as calcium inhibits ATP hydrolysis in fractions enriched in SDH activity<sup>5</sup>. Ca<sup>2+</sup>-ATPase was assayed as the difference between the rates of ATP hydrolysis in the presence and absence of calcium. The standard assay medium contained: 100 mM NaCl, 5 mM MgCl<sub>2</sub>, 3 mM ATP and 50 mM Tris-maleate buffer (pH 7.4). Graded amounts of calcium were added. Below 50  $\mu$ M Ca-EGTA buffers were used. Free Ca<sup>2+</sup> concentrations were calculated by using stability constants and equations given in the literature<sup>8,9</sup>. All solutions used in Ca<sup>2+</sup>-ATPase assays were prepared with water which had been distilled three times in the presence of EDTA. Ca<sup>2+</sup>-free controls contained 4 mM EGTA. Aliquots of 25  $\mu$ l BLM or BBM suspensions (5–15  $\mu$ g protein) were incubated at 25 °C in 0.5 ml assay medium. The reaction was stopped with 0.5 ml 10% trichloroacetic acid and inorganic phosphate was assayed as described elsewhere<sup>10</sup>. Protein, sucrase, (Na<sup>+</sup> + K<sup>+</sup>)ATPase and SDH were assayed as described previously<sup>5,7</sup>.

The kinetics of calcium stimulation were studied in the concentration range  $10^{-7}$  to  $10^{-4}$  M and the results are presented as Eadie-Hofstee plots in Fig. 1. The plots show that the Ca<sup>2+</sup>-ATPase of each plasma membrane fraction has two sites, one of high and one of low affinity for Ca<sup>2+</sup> ions. The  $K_m$  values for the high affinity sites are 0.5 and 1.1  $\mu$ M for BLM and BBM respectively. The  $K_m$  values are similar to those of Ca<sup>2+</sup>-ATPase in red cell membranes and sarcoplasmic reticulum<sup>11,12</sup>. The low affinity sites have  $K_m$  values of 50 and 70  $\mu$ M for BLM and BBM respectively. Optimal activity occurs at Ca concentrations of 0.1 to 0.2 mM and no further stimulation or inhibition is observed at up to 2 mM. As the intracellular Ca concentration is expected to be lower than 10  $\mu$ M, the high affinity sites are of physiological importance. The demonstration of these sites makes the Ca<sup>2+</sup>-ATPase of plasma membranes of rat duodenum a candidate for a calcium pumping mechanism, in analogy to red cell membrane and sarcoplasmic reticulum calcium pumps<sup>11,12</sup>.



In young rats, calcium is actively absorbed in the duodenum and the distribution of  $\text{Ca}^{2+}$ -ATPase activity among the brush border and the basolateral membranes may provide a mechanism for calcium transport<sup>6</sup>. As BBM and BLM are not purified to the same extent, a direct comparison of specific activities is not informative. Therefore, in Table 1 specific  $\text{Ca}^{2+}$ -ATPase activities in BBM and BLM are given after correction for their purification factors. The table shows that below  $5 \mu\text{M}$  there is excess  $\text{Ca}^{2+}$ -ATPase activity in basolateral membranes. As vitamin D alters the permeability of the brush border membrane to calcium<sup>13,14</sup>, an increase in  $\text{Ca}^{2+}$ -influx may result in excess  $\text{Ca}^{2+}$  extrusion across the basolateral membrane due to the excess  $\text{Ca}^{2+}$ -ATPase activity. In order to test this hypothesis, studies have to be done in which  $\text{Ca}^{2+}$  fluxes across BBM and BLM vesicles can be correlated with the amount of ATP hydrolysed by  $\text{Ca}^{2+}$ -ATPase. Unfortunately, resealed BBM vesicles are orientated right-side out with their calcium and ATP binding sides on the inside<sup>15</sup>. The currently available techniques for BLM isolation yield vesicles too leaky to permit ion transport studies<sup>16</sup>.

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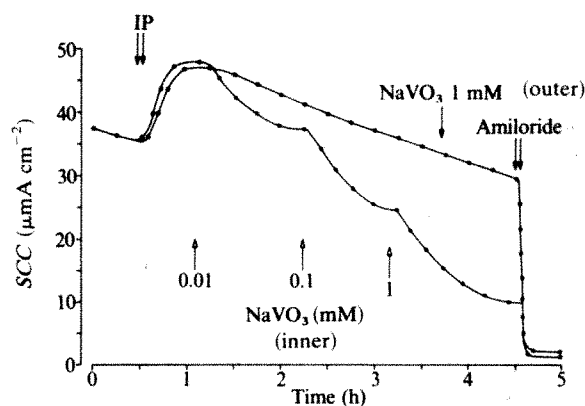
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## Vanadate blocks cyclic AMP-induced stimulation of sodium and water transport in amphibian epithelia

INTEREST in the biological effects of vanadium has grown particularly since Cantley *et al.*<sup>1</sup> identified vanadate as a potent inhibitor of  $(\text{Na}^+ + \text{K}^+)\text{ATPase}$  and Balfour *et al.*<sup>2</sup> reported its striking natriuretic and diuretic properties in the rat. Such findings prompted us to investigate the effects of vanadate on amphibian epithelia, a model widely used for studying, *in vitro*, the membrane transport of sodium and water<sup>3,4</sup>. We found a ouabain-like action of metavanadate on active sodium transport. In addition, we report here a new biological effect of this compound on living cells: the inhibition of cyclic AMP-induced stimulation of transepithelial water flow.

The inhibition of sodium transport was mainly studied in frog skin, but similar results were obtained in toad bladder. Short circuit current (SCC) was taken as a measure of net sodium flux<sup>4,5</sup>. Addition of sodium metavanadate,  $\text{NaVO}_3$ , with V in the



**Fig. 1** Side-specific action of  $\text{NaVO}_3$  on sodium transport across the ventral skin of the frog *R. ridibunda*. The membranes were mounted in double chambers of the Ussing type and bathed by normal Ringer solution on both sides. The two lines indicated by solid circles and open circles correspond to the SCC values of two adjacent segments of the same skin. Exposure of both segments to a  $\beta$ -adrenergic agonist (isoprenaline, IP,  $1 \mu\text{M}$ ) induced similar increments in SCC. Subsequent addition of  $\text{NaVO}_3$  to the inner solution resulted in a progressive, ouabain-like, dose-dependent fall in SCC; in contrast, addition of  $\text{NaVO}_3$  to the outer solution had no effect. The two skin segments were sensitive to amiloride ( $10^{-4} \text{ M}$ ), a specific blocker of the sodium entry sites of the outer surface of the epithelium; this indicates the sodium nature of the SCC in this epithelium.

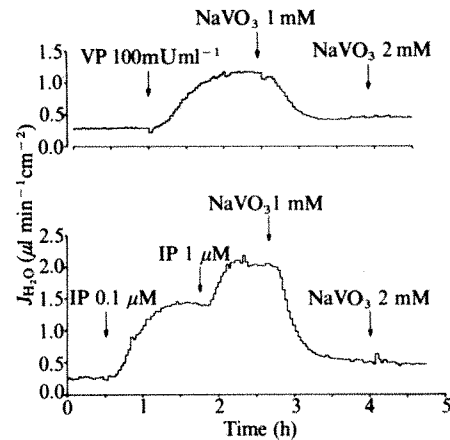
+5 oxidised state, to the solution bathing the internal side of the epithelium, resulted in a dose-dependent decrease in SCC (data not shown). The degree of inhibition and its time course were very similar to those observed in the presence of ouabain or harmaline<sup>6</sup>, two well known inhibitors of the sodium pump. A similar fall in SCC was seen in skins with higher rates of sodium transport due to pre-exposure of the epithelium to natriuretic agents such as catecholamines (Fig. 1), neurohypophyseal hormones, cyclic AMP and theophylline. In addition, pre-exposure of the skins to  $\text{NaVO}_3$  ( $10^{-3} \text{ M}$ ), almost completely prevented the stimulation of SCC by any of the above agents (Table 1). Finally, vanadate was clearly demonstrated to act at only one side of the membrane by the lack of effect of this compound when added to the solution bathing the outer surface of the skin (Fig. 1).

An inhibition of the  $(\text{Na}^+ + \text{K}^+)\text{ATPase}$  of frog skin by  $\text{NaVO}_3$  is the simplest hypothesis to explain the changes in SCC described here<sup>3</sup>. The concentrations of vanadate required to reduce SCC were rather high, but the same applies to ouabain and harmaline, in this tissue<sup>6</sup>. Such relative refractoriness of the enzyme may be related to the presence of an endogenous, ouabain-like inhibitor of the sodium pump, as recently suggested<sup>7</sup>, and/or to an intracellular site of action of vanadate<sup>8</sup> and harmaline<sup>6</sup>.

As the inactivation of the sodium pump affects the last step of the transepithelial movement of sodium, other biological effects of vanadium could remain undisclosed, for example, an interaction between vanadate and the cyclic AMP system of the epithelial cell. We previously observed this type of situation while studying the effects of harmaline in amphibian skin<sup>6</sup>. Therefore, we looked for effects of  $\text{NaVO}_3$  on another transport system, that of water. The osmotic flow ( $J_{\text{H}_2\text{O}}$ ) across the bladder or the skin of toads, *Bufo marinus*, was monitored continuously by means of an automatic, volumetric technique<sup>6,9,10</sup>. Addition of  $\text{NaVO}_3$ , up to  $10^{-3} \text{ M}$ , to the internal medium, did not cause any marked change in  $J_{\text{H}_2\text{O}}$  although a transient but reproducible stimulation was often noted during the first 3 min, particularly in toad bladder. A conspicuous effect was seen, however, when the epithelia were subsequently challenged with a hydrosomotic agent, such as vasopressin, in toad bladder (Table 1) and vasopressin or isoprenaline in toad skin (data not shown).

The increase in  $J_{H_2O}$  induced by these agents was inhibited by vanadate in a dose-dependent manner at concentrations of  $10^{-5}$ – $10^{-3}$  M. Similar results were obtained with theophylline (Table 1). Taken together, these effects of vanadate on  $J_{H_2O}$  could be interpreted as evidence for an inactivation of adenylyl cyclase. If this were the case, one would expect no inhibition of the hydrosmotic effect of exogenous cyclic AMP by  $NaVO_3$  (ref. 3). The experimental results, however, revealed completely the opposite (Table 1). Vanadate also inhibited  $J_{H_2O}$  in epithelia previously exposed to a hydrosmotic agent and thus having a high rate of water flow before  $NaVO_3$  addition; interestingly, the fall in  $J_{H_2O}$  did not go beyond basal water flow (Fig. 2). Finally, as already seen with SCC, the vanadate effect on  $J_{H_2O}$  was side-specific; when added to the outer medium,  $NaVO_3$  did not affect either basal or stimulated  $J_{H_2O}$ .

The biological effects of vanadate on both sodium and water transport described here are relevant to recent physiological<sup>2,11</sup> and biochemical<sup>1,8,12–15</sup> observations made with vanadium. The effects on SCC are consistent with the natriuretic properties<sup>2</sup> and the inhibition of  $(Na^+ + K^+)ATPase$ <sup>1,8,12–14</sup> reported by others. The effects on  $J_{H_2O}$  do not seem to be directly related to the sodium pump inhibition. It has been found in this and other laboratories that ouabain has no appreciable effect on  $J_{H_2O}$  in toad bladder, despite near-maximal reductions of SCC<sup>16</sup>. On the other hand, an interaction between vanadate and the cyclic AMP system would easily explain the  $J_{H_2O}$  data shown in Fig. 2



**Fig. 2** Two examples of the effect of  $NaVO_3$  on cyclic AMP-induced stimulation of water transport ( $J_{H_2O}$ ) across the ventral skin of toads *B. marinus*. The hydrosmotic actions of both vasoressin, VP (upper half), and isoprenaline, IP (lower half), were almost completely blocked by  $NaVO_3$  added to the inner solution. The automatic technique used to record  $J_{H_2O}$  has been described elsewhere<sup>6,9</sup>. The solutions bathing the epithelium were: normal Ringer (inner surface) and Ringer diluted 10-fold (outer surface). Although vanadate inhibited both basal and stimulated SCC, this agent seems to block only the cyclic AMP-induced stimulation of  $J_{H_2O}$  but not basal  $J_{H_2O}$ .

**Table 1** Block by vanadate of stimulated SCC and water flow in amphibian epithelia

	a, SCC (frog skin) ( $\mu A \text{ cm}^{-2}$ )			
	Control		$NaVO_3$ (1 mM)	
	Basal	Peak	Basal	Peak
Oxytocin (10)	17.5	39.0*	6.1	9.1*
50 $\mu M \text{ ml}^{-1}$	$\pm 3.2$	$\pm 4.3$	$\pm 0.9$	$\pm 1.0$
Isoprenaline (10)	15.6	46.6*	6.7	9.0†
1 $\mu M$	$\pm 2.4$	$\pm 4.2$	$\pm 0.8$	$\pm 1.0$
Theophylline (9)	19.1	66.8*	5.8	12.7†
10 mM	$\pm 3.6$	$\pm 6.9$	$\pm 0.8$	$\pm 1.7$
Cyclic AMP (8)	12.7	20.1*	6.3	7.4†
5 mM	$\pm 1.9$	$\pm 2.0$	$\pm 1.2$	$\pm 1.1$
	b, $J_{H_2O}$ (toad bladder) ( $\mu l \text{ h}^{-1} \text{ cm}^{-2}$ )			
	Control		$NaVO_3$ (1 mM)	
	Before	After	Before	After
Vasopressin (9)	1.6	114.0*	5.3	15.6*
50 $\mu M \text{ ml}^{-1}$	$\pm 0.6$	$\pm 14.8$	$\pm 1.3$	2.7
Theophylline (6)	1.8	71.2*	3.6	5.4
10 mM	$\pm 0.4$	$\pm 7.3$	$\pm 1.3$	$\pm 1.7$
				(NS)
Cyclic AMP (6)	7.3	48.5*	4.5	5.6
5 mM	$\pm 1.3$	$\pm 5.9$	$\pm 1.8$	$\pm 2.5$
				(NS)

a, Abdominal skins of frogs *Rana ridibunda* were challenged with different natriuretic agents. In each experiment two segments of the same skin were used, one of which had been pre-exposed to  $NaVO_3$  added to the internal solution. Values in the first and third columns correspond to SCC recordings before addition of the agent indicated on the left; values on second and fourth columns correspond to peak responses obtained after exposure to the same agent. b, A similar protocol was applied to quarter-bladders of toads *Bufo marinus* to determine the effect of vanadate on cyclic AMP-dependent stimulation of water flow ( $J_{H_2O}$ ). Values represent cumulative  $J_{H_2O}$  (for 60-min periods), before and after addition of the hydrosmotic agent indicated on the left. The first two columns show results using the control quarter-bladders, the last two, paired quarter-bladders exposed to vanadate. Similar results were obtained in toad skin. Only the skins were challenged with isoprenaline, as the urinary bladder lacks  $\beta$ -adrenergic receptors. All values are mean  $\pm$  s.e.m. The number of paired studies is indicated in parentheses. Statistical analysis was done by using the paired *t*-test. Significance of *P* values is indicated by NS (non-significant), \* $<0.001$ , † $<0.01$  and ‡ $<0.05$ . On exposure to  $NaVO_3$ , fractional increases in SCC and cumulative  $J_{H_2O}$  values were significantly different with respect to control values for all the natriuretic and hydrosmotic agents tested.

and Table 1. Without obviously excluding an effect of vanadate on adenylyl cyclase<sup>15</sup>, a site of action beyond cyclic AMP generation must be postulated to account for the inhibition of the effect of exogenous cyclic AMP. Two lines of evidence suggest that an ATPase linked to mechanochemical proteins might be such a target: (1) the involvement of microtubules and microfilaments in epithelial water transport<sup>17–19</sup> and (2) the inhibition of dynein-ATPase by vanadate<sup>20–22</sup>. Further work is needed to elucidate this mechanism, which possibly contributes to the huge diuresis observed in the rat. Overall, our results indicate that the amphibian epithelium is a useful model for distinguishing between the many biological actions of vanadium.

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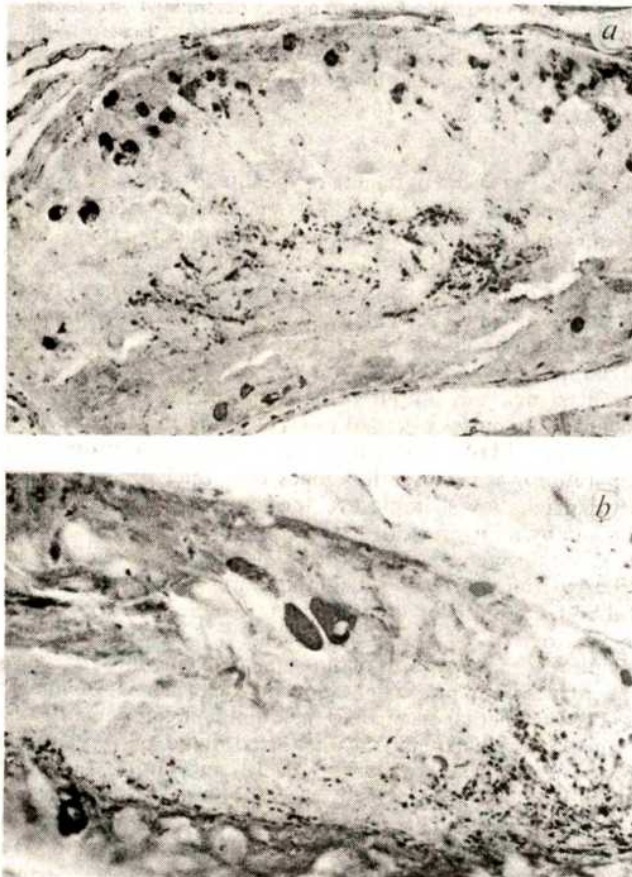
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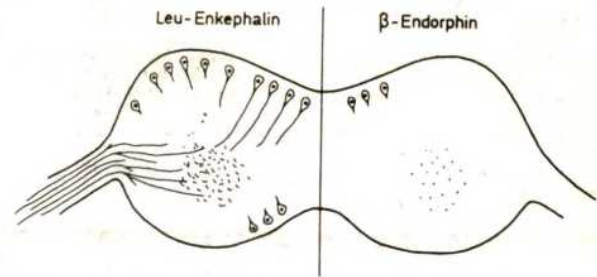
## Neuronal localisation of immunoreactive enkephalin and $\beta$ -endorphin in the earthworm

SINCE the report by Simantov *et al.*<sup>1</sup> invertebrates have been thought to lack endogenous opiates and opiate receptors. As we have previously observed peptidergic neurones containing immunoreactive pancreatic polypeptide or vasoactive intestinal peptide in the earthworm<sup>2</sup>, we decided to search for, and report here our finding of, nerves storing immunoreactive enkephalin or  $\beta$ -endorphin in this species.

Various segments of earthworms (*Lumbricus terrestris*) were cut and frozen in a propane-propylene mixture cooled to the temperature of liquid nitrogen. After freeze-drying the specimens were fixed in formaldehyde vapour at 80 °C and embedded in paraffin *in vacuo*. Sections, 5  $\mu$ m thick, were deparaffinised and used for the immunohistochemical demonstration of enkephalin or  $\beta$ -endorphin, using either the indirect immunofluorescence technique<sup>3</sup> or the PAP technique of Sternberger<sup>4</sup>. The enkephalin antiserum used was raised against synthetic Leu-enkephalin coupled to bovine serum albumin. The characteristics of this antiserum are described elsewhere<sup>5,6</sup>. The  $\beta$ -endorphin antiserum (Milab) was raised as follows. Synthetic human  $\beta$ -endorphin ( $\beta$ -lipotropin (61–91); Peninsula) was emulsified in Freund's complete adjuvant. It was injected intracutaneously at multiple sites in rabbits, at a dose of 100  $\mu$ g of  $\beta$ -endorphin per rabbit. Two booster injections of 20  $\mu$ g were given at 3-monthly intervals. The third booster injection of 100  $\mu$ g  $\beta$ -endorphin was given a further 3 months later, and sera



**Fig. 1** Cerebral ganglion of the earthworm. Circumpharyngeal connective to the right. *a*, Many Leu-enkephalin-immunoreactive nerves and cell bodies (PAP staining). Note the peripheral localisation of the cell bodies and the central localisation of the nerves.  $\times 300$ . *b*,  $\beta$ -Endorphin-immunoreactive nerves and cell bodies.  $\times 400$ .



**Fig. 2** Cross-section of the cerebral ganglion of the earthworm to show the distribution and relative frequency of enkephalin- and  $\beta$ -endorphin-immunoreactive nerves and cell bodies. Some of the nerves seem to reach the circumpharyngeal connectives. Transsected nerves are indicated by dots.

were collected 2 months after this. The specificity of the  $\beta$ -endorphin antiserum (diluted 1:10,000) was tested in a radioimmunoassay system. It did not cross-react ( $<0.1\%$ ) with  $\alpha$ -MSH (ACTH (1–13)),  $\beta$ -MSH ( $\beta$ -lipotropin (41–58)), Met-enkephalin ( $\beta$ -lipotropin (61–65)),  $\alpha$ -endorphin ( $\beta$ -lipotropin (61–76)),  $\gamma$ -endorphin ( $\beta$ -lipotropin (61–77)),  $\beta$ -lipotropin (60–65),  $\beta$ -lipotropin (39–45), ACTH (1–24), ACTH (34–39) or ACTH (4–11).

The enkephalin antiserum was used in a dilution of 1:40 (immunofluorescence) or 1:400 (PAP staining), and the  $\beta$ -endorphin antiserum in a dilution of 1:20 or 1:200, respectively. Controls included application of antiserum inactivated by addition of excess Leu-enkephalin or  $\beta$ -endorphin (Peninsula) (10  $\mu$ g per ml diluted antiserum). Many neuronal cell bodies of different sizes and nerve fibres displaying intense enkephalin immunoreactivity were found in the cerebral ganglion. Characteristically, the cell bodies were located in the periphery whereas the nerve fibres occurred in the central part (Figs 1*a*, 2). Immunoreactive nerves were numerous within the cerebral ganglion and extended into the circumpharyngeal connectives. Immunoreactive nerve cell bodies and a few nerves were occasionally found in the subpharyngeal ganglion and in the abdominal ganglia. Cell bodies displaying  $\beta$ -endorphin immunoreactivity had a distribution similar to that of the enkephalin immunoreactive ones. Generally, the  $\beta$ -endorphin cells seemed to be larger in size and much fewer in number (Figs 1*b*, 2). They were clearly distinct from the cell bodies containing immunoreactive enkephalin.  $\beta$ -Endorphin-immunoreactive nerves were scattered in the central part of the cerebral ganglion but were absent from the circumpharyngeal connectives. In the subpharyngeal ganglion and the abdominal ganglia immunoreactive cell bodies and nerves were rare.

The results obtained indicate that enkephalin and  $\beta$ -endorphin, or closely related peptides, exist in the neurones of the earthworm. Hence, this suggests that opiate receptors may also be found in this species. In view of the present findings the belief that endogenous opiates and opiate receptors do not exist in invertebrates may have to be reconsidered.

While this manuscript was being prepared Rémy and Dubois<sup>7</sup> described a few  $\alpha$ -endorphin-immunoreactive cell bodies in the subpharyngeal ganglion of the earthworm. They did not find  $\beta$ -endorphin immunoreactivity.

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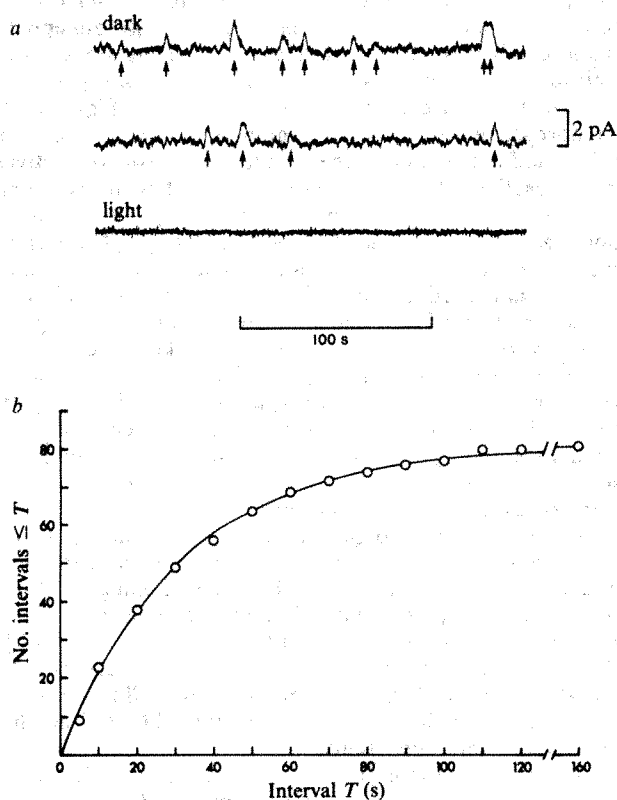
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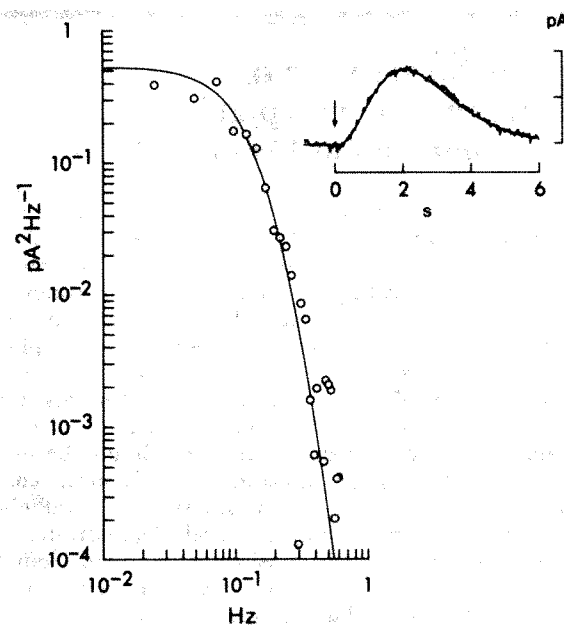
## Thermal activation of the visual transduction mechanism in retinal rods

INVERTEBRATE photoreceptors show electrical changes which apparently result from isomerisation of single rhodopsin molecules by light or thermal energy<sup>1-3</sup>. Observation of corresponding phenomena in vertebrates has been prevented by intercellular electrical coupling, which averages membrane potential over many photoreceptors<sup>4-6</sup>. Recently, however, recordings of membrane current from individual rod outer segments have revealed responses to single photons<sup>7,8</sup>. Here we report that similar electrical events occasionally occur in darkness, perhaps because of thermal isomerisation of rhodopsin.

Membrane current was recorded from single rod outer segments in toad (*Bufo marinus*) retina using a method described previously<sup>9</sup>. Pieces of thoroughly dark-adapted retina were



**Fig. 1** *a*, Sample records of rod outer segment current in darkness (two upper traces, sequential records) and bright light (bottom trace). Arrows below traces indicate times at which current exceeded a criterion level 0.5 pA above the baseline; these times are taken as occurrences of discrete events, discussed in the text. Using this method a total of 82 events were counted over a period of 2,631 s. In light the photocurrent reached a saturating amplitude of 19 pA and no events were evident in the observation period of 1,106 s. Records were low-pass filtered at 5 Hz with a 6-pole filter. Temperature 22 °C. *b*, Cumulative distribution of intervals between successive events in the same cell shown in *a*. Circles are experimental points; curve drawn according to equation (1) in text with  $\tau = 32$  s,  $N = 81$ .



**Fig. 2** Comparison of power spectral densities of dark events and single photon response in a rod.  $\circ$ , Difference power spectrum of dark events obtained as spectrum of all dark sweeps minus spectrum of dark sweeps not containing events. Experimental spectra based on 43 sweeps, each 40.96 s long, digitised at 20-ms intervals after low-pass filtering at 15 Hz. Continuous spectrum calculated from the Fourier transform of equation (2) in the text, with  $\alpha = 1.33$  s<sup>-1</sup>. Vertical scaling of theoretical spectrum, chosen to give best fit to points, corresponds to a mean rate of 0.022 s<sup>-1</sup> assuming dark events had the same amplitude (1 pA) as this cell's single photon response. Event frequency determined by counting was 0.023 s<sup>-1</sup>. Temperature 21.6 °C. Inset, jagged curve is the average of 80 responses of the same cell to dim flashes delivering an average of 0.8 effectively absorbed photons per flash. Flash timing shown by arrow. Smooth curve is equation (2), with  $\alpha = 1.33$  s<sup>-1</sup>.

placed in a chamber containing oxygenated Ringer and viewed in a compound inverted microscope. Retinal isolation and subsequent procedures were carried out under infrared illumination with an IR/visible image converter. Under visual control the outer segment of a rod in one of the pieces of retina was drawn by gentle suction into the tip of a glass micropipette. A low-noise amplifier connected to the inside of the pipette gave an output voltage proportional to membrane current. This current signal, the output of a light stimulus monitor, and synchronising pulses were led to an FM tape recorder for later analysis in a PDP 11/34 computer. For 'dark' recordings the preparation was enclosed in a black box which attenuated the very dim light (low scotopic level for humans) in the experimental room by a factor of 10<sup>6</sup>. Heated or cooled water could be circulated through a jacket surrounding the central Ringer pool in the experimental chamber; temperatures were measured with a calibrated thermistor about 0.5 mm from the tip of the recording electrode.

Figure 1a shows records of outer segment current in darkness (upper traces) and bright light (bottom trace). The noise in light was of instrumental origin, and the dominant component had a magnitude predicted by the measured value of the leakage resistance between electrode wall and outer segment membrane. The excess fluctuation in the dark records consisted of two components: (1) occasional discrete events (indicated by arrows below dark traces) of about 1 pA amplitude, and (2) a continuous small-amplitude fluctuation, not discussed further here. The discrete events resembled the average response to a single photon and in this cell were estimated to occur at a mean rate of 0.031 s<sup>-1</sup>. In the experimental conditions, however, the expected rate of photoisomerisations from stray light was at least two orders of magnitude below the observed event rate. This suggests that the events were not triggered by photons

but reflected spontaneous activation of the transduction mechanism.

If random independent excitations occurred in the rhodopsin molecules or intracellular disks of the outer segment the intervals between events should be exponentially distributed. Figure 1b compares an experimental interval histogram with this prediction. The continuous curve was drawn according to

$$n = N(1 - \exp(-T/\tau)) \quad (1)$$

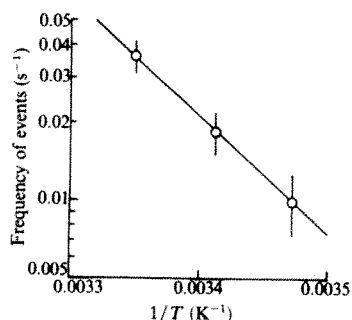
where  $n$  is the number of intervals less than or equal to  $T$ ,  $\tau$  is the average interval, and  $N$  is the total number of intervals observed. The satisfactory agreement between experimental results and equation (1) supports the interpretation that the occurrence of events is a Poisson process describable by the single parameter,  $\tau$ , the average interval between events. Similar agreement between experimental results and theoretical curves was observed in seven other experiments, with a range of values for  $\tau$  of 76–17 s at temperatures of 15–26 °C.

The amplitude of the dark events was similar to the amplitude of the same cell's single photon response. To compare the kinetics of dark events and single photon responses we examined their power spectra; previous work<sup>8</sup> has shown that the current noise evoked by dim steady light has the spectrum of the average single photon response. The spectrum of the dark events was obtained as the spectrum of all dark sweeps minus the spectrum of dark sweeps not containing events. The average single photon response in each experiment was fitted by the relation<sup>9</sup> (Fig. 2 inset)

$$r(t) = k(\alpha t)^3 e^{-\alpha t} \quad (2)$$

where  $r$  is the photocurrent as a function of time  $t$  after photon absorption,  $k$  is a scaling constant and  $\alpha$  is a rate constant characteristic of the cell. This equation would apply if photoisomerisations cause electrical action by a process involving a series of four first-order delays<sup>10,11</sup>. The points in Fig. 2 are the spectrum of the dark events, and the curve is the calculated spectrum of the same cell's single photon response. Similarly close agreement between observed and calculated spectra was seen in experiments on eight other cells. The ability of the equation for the flash response to predict the spectrum of the dark events suggests that they are triggered by fluctuations at the input of the cascade. A simple explanation would be that the dark events result from a thermal configuration change in rhodopsin itself.

Figure 3 shows the temperature dependence of the rate of occurrence of dark events. The slope of the Arrhenius plot gives an activation energy  $E_a$  of 20.9 kcal mol<sup>-1</sup> for the thermal excitation process. In similar experiments on five cells the value of  $E_a$  was 23.2 ± 3.2 kcal mol<sup>-1</sup> (mean ± s.d.). The mean frequency of events in these experiments was 0.021 s<sup>-1</sup> at 20 °C.



**Fig. 3** Arrhenius plot of frequency of occurrence of dark events in a rod (log scale) against reciprocal absolute temperature. Detection of events in the continuous background noise was facilitated by digitally processing the current records with a 'matched filter' (ref. 15). In this procedure equation (2) was fitted to the rod's single photon response and then cross-correlated with the dark recordings. Vertical bars are based on the square root of the number of events counted. Slope of linear regression line corresponds to activation energy  $E_a$  of 20.9 kcal mol<sup>-1</sup>.

Assuming that the rod outer segment contains  $2 \times 10^9$  rhodopsin molecules and that thermal fluctuations in rhodopsin cause the dark events, the estimated rate constant for thermal excitation of rhodopsin is  $10^{-11}$  s<sup>-1</sup>. This corresponds to a molecular half life of the order of 1,000 yr. Additional parameters for the thermal excitation of rhodopsin at 20 °C were calculated as: Gibbs free energy of activation  $\Delta G^\ddagger = 31.9 \pm 0.13$  kcal mol<sup>-1</sup>, entropy of activation  $\Delta S^\ddagger = -31.7 \pm 11.2$  e.u.

Ashmore and Falk<sup>12</sup>, by analysis of membrane noise in neurones postsynaptic to dogfish rods, estimated values of rate constant and  $\Delta G^\ddagger$  similar to our own but found  $E_a$  to be 36 kcal mol<sup>-1</sup> and  $\Delta S^\ddagger$  to be +13 e.u. The differences may reflect different experimental methods. Chemical studies show that thermal isomerisation of 11-*cis* retinal in aqueous digitonin solution<sup>13</sup> is associated with a small negative activation entropy of -12.5 e.u. and  $E_a$  of 24.5 kcal mol<sup>-1</sup>, whereas for thermal denaturation of cattle rhodopsin in rod fragments Hubbard<sup>14</sup> found  $\Delta S^\ddagger = +214$  e.u. and  $E_a = 100$  kcal mol<sup>-1</sup>. Furthermore, extrapolation to 20 °C gives a rate constant for denaturation in rod fragments three orders of magnitude below the excitation rate constant derived here. Isomerisation is thus the more likely trigger of dark events. In a variety of solvents, however, derived rate constants for thermal isomerisation of 11-*cis* retinal alone are two to three orders of magnitude larger than the spontaneous excitation rate constant. This implies that linkage with opsin stabilises the 11-*cis* configuration of the chromophore.

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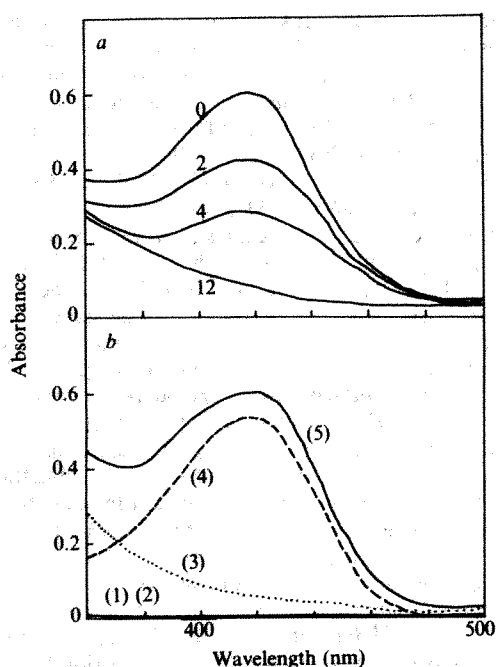
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## Photoinduced electron transport across phospholipid wall of liposome using methylene blue

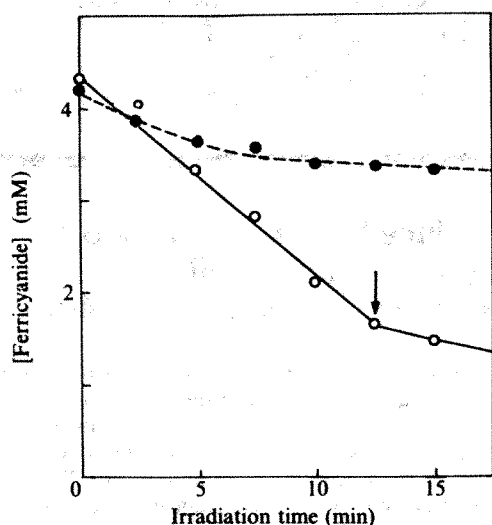
PHOTOSENSITISED electron transport across membranes is a useful model reaction for photosynthesis. We have previously reported<sup>1</sup> the photoredox reaction in/on liposomes. Other similar studies have used chlorophyll as a sensitizer<sup>2,3</sup>, but this compound is easily broken and difficult to purify. The surfactant analogues of metal complexes (particularly tris-(2,2'-bipyridine) Ru<sup>2+</sup>)<sup>4–6</sup> are also widely used, but with such photosensitisers which possess long alkyl chains, electron transport does not occur in the absence of electron carriers such as quinones or carotenes. We report here the occurrence of photosensitised electron transport across the single phospholipid wall of the



**Fig. 1** *a*, Progressive spectral changes with time of irradiation; [MB] = 0.01 mM; [ASc] = 0.008 M. Numbers on each curve indicate the irradiation time (min)., UV spectra of each component in Tris buffer; (1) [MB] = 0.01 mM; (2) [ASc] = 0.008 M; (3) dispersed lecithin; (4) [potassium ferricyanide] = 4 mM; (5) 1 + 2 + 3 + 4.

liposome using methylene blue as both a sensitiser and an electron carrier. This is the first report of photosensitised electron transport across a membrane using a dye not only as a photosensitiser but also as an electron carrier.

The reaction was carried out in the single lamella liposome system. The liposome system, containing water-soluble electron acceptor ( $K_3Fe(CN)_6$ ) only in the inner aqueous phase of the vesicle and water-soluble electron donor (ascorbic acid) only in the outer aqueous phase, was prepared in the following way. Lecithin (isolated from egg-yolk<sup>7</sup>) was dispersed in 1 M  $K_3Fe(CN)_6$  solution buffered with 1 M Tris-Cl and 0.1 M KCl, pH 7.5. The dispersion was then ultrasonicated under  $N_2$  gas and the undispersed phospholipids were removed by gel filtration over a column of Sephadex G-50 equilibrated with the buffer solution. Then a given amount of methylene blue



**Fig. 2** Change in ferricyanide concentration with time of irradiation. O, [MB] = 0.008 mM, [ASH] = 8 mM; [ASH] > [ferricyanide]. ●, [MB] = 0.008 mM, [ASH] = 0.08 mM; [ASH] < [ferricyanide]. Arrow indicates the time at which the light was cut off.

was added. After being degassed by passing  $N_2$  gas, the dispersion was added to a solution of ascorbic acid at a final concentration of 8 mM. As soon as the solution was mixed, it was sealed in a Pyrex tube with a UV cell on the side arm (or sealed in a UV cell as a control). The photoreaction was carried out immediately by irradiating the solution with a super-high-pressure mercury lamp. Light of wavelengths shorter than 460 nm was completely removed by the use of a UV-46 filter.

In these conditions methylene blue was only very slightly reduced by ascorbic acid without light. However, when the light was irradiated, the absorbance at 420 nm decreased rapidly (Fig. 1*a*). Neither ascorbic acid nor methylene blue absorbs the light at 420 nm (Fig. 1*b*), and the absorption of lecithin was constant even with irradiation. Therefore, the decrease in the potassium ferricyanide concentration could be measured by measuring the decrease in the absorbance at 420 nm.

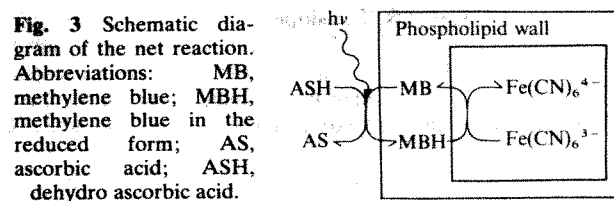
**Table 1** Relative reaction rates in different conditions

	Relative reaction rates	
	Irradiated	Non-irradiated
(A) Complete system	1	0.05
(B) A - ascorbic acid	0.01	0
(C) A - methylene blue	0	0

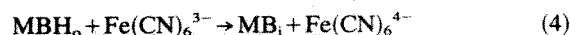
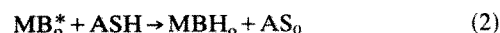
Complete system: methylene blue, 0.01 mM; ascorbic acid, 0.01 M.

Figure 2 shows the decrease in the ferricyanide concentration with time of irradiation. When the ascorbic acid concentration is higher than that of potassium ferricyanide, the reaction proceeds until all the ferricyanide is completely reduced. If the light is cut off, the reaction stops. When the ascorbic acid concentration is lower than that of potassium ferricyanide, the reaction stops after all ascorbic acid has been oxidised. These results confirm that ferricyanide is reduced.

As shown in Table 1, the reduction of  $K_3Fe(CN)_6$  did not occur in the absence of methylene blue or in the absence of ascorbic acid, even when the system was irradiated. The reaction was almost undetectable without light. This shows that either direct reduction of  $K_3Fe(CN)_6$  by ascorbic acid or the penetration of ascorbic acid did not occur. However, ascorbic acid cannot exist in the reduced form in the inner aqueous phase, and furthermore, the reduction rate increased with increase in the concentration of methylene blue.



From these results, the reaction can be summarised as proceeding through the following steps (see also Fig. 3).



where  $MB_0$  and  $MBH_0$  are methylene blue and reduced methylene blue in the outer aqueous phase,  $MB_i$  is methylene blue in the inner aqueous phase, AS and ASH are ascorbic acid and dehydroascorbic acid, and \* indicates an excited state.

We do not understand exactly how step (3) occurs, but it is clear that the electron is transported from ascorbic acid in the outer aqueous phase to  $K_3Fe(CN)_6$  in the inner aqueous phase, and that this electron transport is induced by the photo-irradiation. We are continuing our investigation of the transport process.



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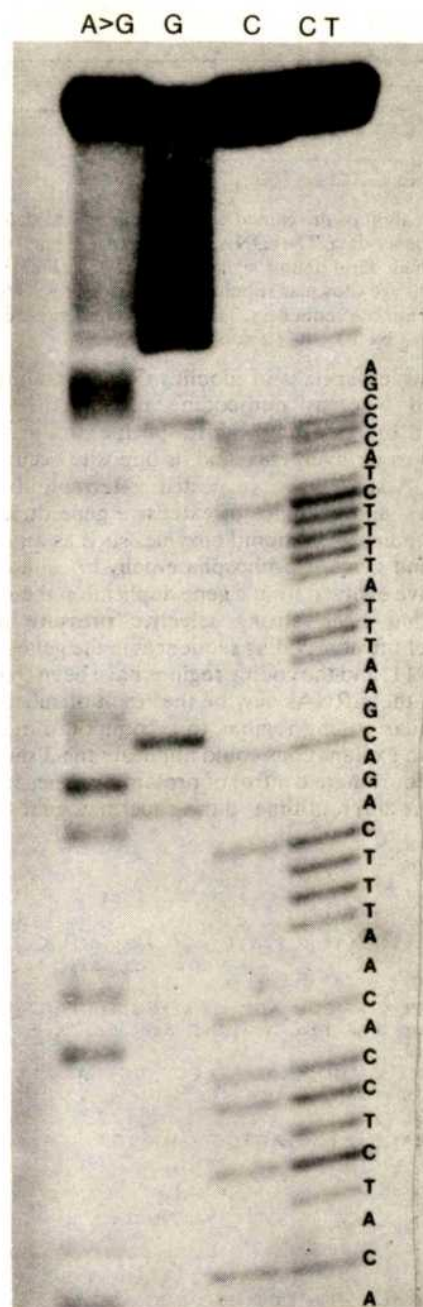
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## Sequence divergence of rainbow trout protamine mRNAs; comparison of coding and non-coding nucleotide sequences in three protamine cDNA plasmids

SPERMATOGENESIS in the rainbow trout (*Salmo gairdnerii*) is associated with the onset of synthesis of the protamines<sup>1,2</sup>, a family of small, highly basic proteins consisting of 32 or 33 residues, of which approximately two-thirds are arginine<sup>3</sup>. By displacing the histones, the protamines have a unique role in packaging sperm DNA. The biosynthesis of the protamines has been studied in some detail, and polyadenylated protamine mRNA of about 290 nucleotides has been isolated from trout testis<sup>4,5</sup> and shown by *R<sub>0</sub>t* analysis to be made up of three to four components<sup>5,6</sup>. More recent work suggests that there are about six different protamine genes per haploid genome<sup>7</sup>. In contrast to the interspecific conservation of histone sequences, the observed rate of protamine sequence divergence between the closely related clupeid fishes such as trout, salmon and herring, is very high, and there is evidence that protamine genes have arisen following a doubling of the ancestral fish genome. Comparison of the cloned complementary DNA sequences and ultimately of the genes themselves will help to elucidate the molecular events involved in the evolution of these proteins. To study the chromosomal organisation of these genes and the control of their expression during spermatogenesis, I have constructed cDNA clones using purified poly(A)<sup>+</sup> mRNA as starting material. Here, the sequences of three of these recombinants are compared, revealing an unexpected pattern of divergence in the coding and non-coding regions, as well as a mutational 'hot-spot' corresponding to the major phosphorylation site of the protamines.

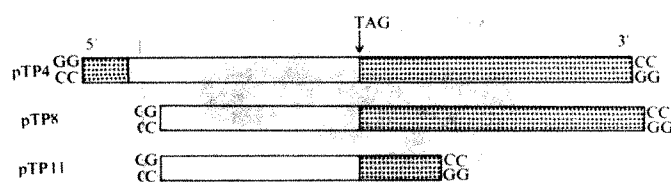
The sequences of the cloned cDNA fragments were determined by the methods of Maxam and Gilbert<sup>8</sup>. Figure 1 is a representative sequence ladder and Fig. 2 shows the extent of the cloned sequences aligned with reference to the functional chain terminator codon TAG. pTP4 contains a complete coding sequence, as well as 5'- and 3'-non-coding regions, and pTP8 and pTP11 have incomplete coding sequences; the fact that they both start at the same point may reflect some secondary structure of the parent mRNA. Both pTP4 and pTP8 contain the hexanucleotide corresponding to A<sub>2</sub>UA<sub>3</sub> which seems to be a general feature of eukaryotic poly(A)<sup>+</sup> mRNAs<sup>9</sup>; the presence of this hexanucleotide in protamine mRNA has been reported previously<sup>10</sup>.

The coding regions of the three clones were located by reference to the published amino acid sequences for rainbow trout protamines<sup>3</sup> (Fig. 3a, b). When these coding sequences are compared (Fig. 4) there is a striking homology between pTP8 and pTP11, with only one substitution in 80 nucleotides. The coding region of pTP4 is markedly different from that of the other two, and there is only a 78% homology when the



**Fig. 1** Poly(A)<sup>+</sup> mRNA was purified from pooled rainbow trout testes, double-stranded cDNA synthesised and inserted into the *Pst*I site of plasmid pBR322 using dG · dC homopolymer tailing, and transfected into the disabled host strain  $\chi$ 176 (ref. 5). Recombinants containing protamine cDNA sequences were identified by liquid hybridisation to cDNA synthesised from purified protamine mRNA<sup>5</sup>. Three recombinants, pTP4, pTP8 and pTP11, were chosen for sequence analysis. All work involving recombinant DNA was carried out in a Category II facility in accordance with GMAG recommendations. This figure shows a sequence gel of the 3'-non-coding region of pTP8. *Iap*I-cleaved pTP8 insert DNA was labelled at this cleavage site using T4 polymerase, and the unique end-labelled fragments separated on a 10% acrylamide gel and sequenced by the method of Maxam and Gilbert<sup>8</sup>. The gel shown is 12% acrylamide, 7M urea.

sequences are aligned, taking into account the putative deletion in pTP4. In contrast, a comparison of the non-coding regions of these cDNA fragments shows a complete reversal of these homology relationships (Fig. 3a). Thus, from the chain termination signal to the 3'-end of the segment of pTP11 which has been cloned, pTP8 and pTP11 differ by over 20% in nucleotide sequence; over this same region pTP4 and pTP11 are identical. This is unexpected because at least where it has been



**Fig. 2** The extent of the cloned cDNA fragments as determined from the sequence data. The cDNA fragments are aligned using the functional chain termination signal codon TAG (Fig. 3). Non-coding regions are shown as stippled and the polarity corresponds to that of the mRNA sequences. The cDNA fragments are flanked by dG·dC homopolymer linkers.

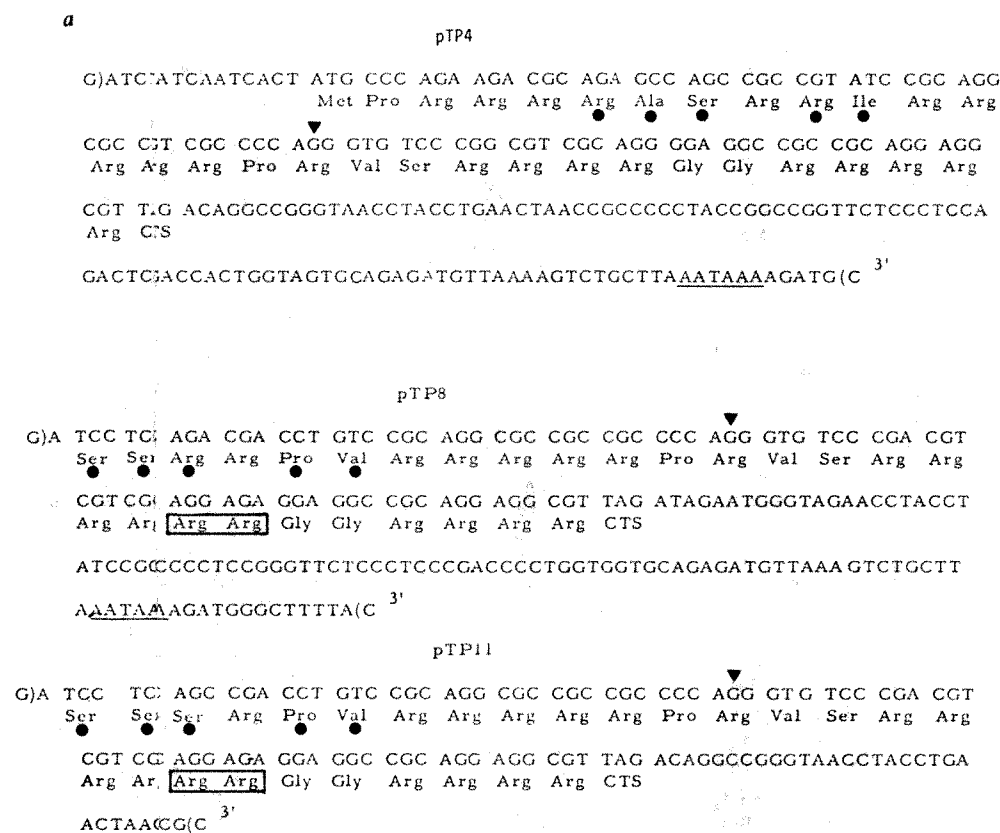
examined between species in globin mRNAs, coding regions are conserved whereas non-coding regions diverge more rapidly<sup>11-13</sup>. In the case of the intraspecific protamine clones both this pattern of divergence and its opposite occur.

Ohno and Atkin<sup>14</sup> have suggested a tetraploid origin of salmonid fishes, and evidence of extensive gene duplication is provided by studies of salmonid enzymes such as malate dehydrogenase<sup>15</sup> and glucose-6-phosphate dehydrogenase<sup>16</sup>. If the protamines have evolved from a gene duplication then since this event there has been strong selective pressure favouring conservation of the non-coding sequences in the genes cloned in pTP4 and pTP11, and the coding regions have been free to drift. Alternatively, the mRNAs may be the result of splicing events linking dissimilar coding regions to a common 3'-non-coding sequence. Both explanations could implicate the 3'-non-coding region in the coordinate control of protamine genes which may be expressed at different times during spermatogenesis<sup>17</sup>.

Within the 3'-non-coding regions of pTP4 and pTP8 the longest run of conserved sequence is one of 22 bases including the hexanucleotide AATAAA. Substantial homology has been found in this region between rabbit, human and mouse  $\beta$ -globin nucleotide sequences<sup>18,19</sup>, suggesting that these flanking sequences, as well as the AATAAA, may be involved in some common control function.

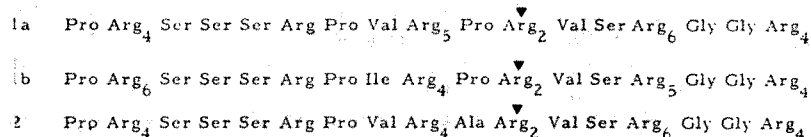
None of the predicted amino acid sequences corresponds exactly to any of those published<sup>3</sup> (Fig. 3a, b). One explanation for this is that the cloned sequences correspond to minor variants within the protamine population, as the mRNA was isolated from pooled testes. However, a more likely explanation is that some error occurred in the amino acid sequencing. The pattern of amino acid substitutions found in the predicted sequences suggests there is a mutational hot spot in residues 6-11 of the N-terminal region (where the N-terminal methionine residue of pTP4 = residue 1), as five out of six residues have changed in this region by base transition and sequence rearrangement. This hot spot is present to a lesser extent in the clupeines<sup>20,21</sup> but is not seen in the sequences reported by Ando and Watanabe<sup>3</sup>. It may be significant that this region contains three of the four serine residues phosphorylated by cyclic AMP-dependent protein kinase during histone displacement and nucleoprotamine assembly<sup>22,23</sup>.

The high frequency of arginine and serine in the protamines makes their mRNAs interesting for the study of codon usage. Davies *et al.*<sup>24</sup> reported that there is no preferential use of any arginine codon in protamine mRNA and that the dinucleotide CpG is under-represented, as in vertebrate DNA<sup>25</sup> and mRNA<sup>26</sup>, despite the fact that it is present in four of the six



**Fig. 3** Complete nucleotide sequences of the three protamine cDNA fragments carried in pTP4, pTP8 and pTP11, showing the encoded amino acid sequences. The cDNA fragments are flanked by dG·dC homopolymer linkers which are represented here by G)....(C'. CTS marks the functional chain termination signal. Amino acid differences between the cDNA encoded sequences are indicated by ●. Residues in pTP8 and pTP11 corresponding to the deletion in pTP4 are boxed. ▼ Indicates the positions at which there is a common difference between the cDNA encoded and the published sequences.

**b**





pTP4<sup>5'</sup> ACAGCCAGCGCGCGTATCCGCAGGCGCGCTCGCCCCAGGGTGTCCCGCGCGTCC C  
 pTP8 TCCGCCAGCGACCTGTCCGCAGGCGCGCGCGCCCCAGGGTGTCCCGACGTCTCGC  
 pTP11 TCCTCCAGCGCGACCTGTCCGCAGGCGCGCGCGCCCCAGGGTGTCCCGACGTCTCGC

pTP4 AGGGGAGGCGCGCGCAGGAGGCGTTAG/ ACAGGCGGGTA ACCTACCTGAACCT  
 pTP8 AGGAGAGGAGGCGCGCAGGAGGCGTTAG/ ATAGAA TGGGTAGAACCTACCTGACCT  
 pTP11 AGGGGAGGCGCGCGCAGGAGGCGTTAG/ ACAGGCGGGTA ACCTACCTGAACCT

pTP4 AACCGCCCCCTACCGGCGCGGTCTCCCTCCAGACTCGACCACTGGTGGTGCAGAGGT  
 pTP8 ATCCGCGCGCT CCGGGTCTCCCTCC CGACCCCTGGTAGTGTAGAGAT  
 pTP11 AACCG(C<sup>3'</sup>)

pTP4 GTTAAAGTCTGCTTAAATAAAAGATG(C<sup>3'</sup>)  
 pTP8 GTTA AAGTCTGCTTAAATAAAAGATGGGCTTTTA(C<sup>3'</sup>)

**Fig. 4** Composition of the shared nucleotide sequences of the protamine cDNA fragments carried in pTP4, pTP8 and pTP11. Coding sequences are separated from non-coding by /. Base transitions are represented by ●. Gaps in the sequence correspond to putative deletions required to give maximum alignment of sequences. Note that 29 nucleotides have been omitted from the 5'-end of pTP4.

possible arginine codons. The sequence data present a completely different picture. The arginine codons CGC and AGG are significantly over-represented, as is the serine codon TCC. CpG frequency in the coding regions of all three clones is that statistically expected from their G+C content and the dinucleotide appears exclusively in the arginine codon series CGX, which accounts for well over half the arginine codons in these sequences. However, in the 3'-non-coding regions, CpG frequency is depressed by about 50% compared to that expected. Methylation of DNA in eukaryotes seems to occur predominantly as 5-methyl CpG<sup>27</sup>, and it has been suggested<sup>12</sup> that this may be the reason the dinucleotide is under-represented, for in bacteria at least, 5-methyl C residues are mutational hot spots probably due to their spontaneous deamination to thymine<sup>28</sup>. The base transitions in these protamine cDNA fragments, however, fail to implicate CpG in sequence diversion, as there is only one instance of a C to T transition with G as its 3'-neighbour. C to T transitions in the first base of the arginine coding series CGX would generate the codons for cysteine and tryptophan or the chain terminator TGA. The presence of Cys and Trp in protamines could interfere with their function, thus selecting against C-T transitions in the DNA coding regions and it is significant that these amino acids are absent from both trout and the closely related herring protamines (clupeines)<sup>20,21</sup>.

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## Infectivity in mouse fibroblasts of polyoma DNA integrated into plasmid pBR322 or lambdoid phage DNA

THERE has been much speculation on the possible hazards of hybrid DNA technology<sup>1</sup>. An important question in this context is whether a viral DNA, covalently linked to a vector, can be transferred from within a bacterium into an animal cell, either *in vitro* or *in vivo*, and initiate viral infection. As a first step, it is necessary to determine whether a hybrid containing eukaryotic viral DNA can, on penetration into a permissive cell, give rise to virus formation, as is the case for hybrids containing a prokaryotic viral sequence<sup>2</sup>. As part of a risk-testing programme sponsored by EMBO, this question was investigated with mouse polyoma virus DNA. Polyoma virus will grow readily in mice; infection can be initiated by a single infectious particle as well as by naked viral DNA, and can be easily detected by monitoring the production of anti-viral antibodies. Virus production, and not tumour formation, was chosen as a criterion for biological activity because although polyoma virus will transform cells *in vitro*, tumours are produced *in vivo* only when large quantities of virus are inoculated into immunologically immature or immunosuppressed animals. We describe here the construction and characterisation of various plasmid pBR322 and phage  $\lambda$  derivatives containing polyoma DNA and their infectivity towards mouse fibroblast cells. The only recombinant molecules so far obtained which were infective as intact molecules were plasmids with dimeric polyoma DNA inserts.

Hybrid plasmid molecules were made with DNA from a readily identifiable mutant of polyoma<sup>3</sup> (which lacked an *Hha* restriction site) by digestion with restriction enzymes *Eco*RI or *Bam*HI, each of which cleaves at a single site, and ligation to appropriately cleaved pBR322. The molar ratios of viral to plasmid DNA used were between 2:1 and 1:1. *Escherichia coli* HB101 was transformed with the recombinant DNA, and colonies containing polyoma DNA were identified by *in situ* hybridisation<sup>4</sup> to <sup>32</sup>P-labelled polyoma RNA. Of 28 hybrid plasmids made by joining the constituent DNAs by their *Bam*HI sites, two had electrophoretic mobilities in agarose gels (0.5 and 0.52 relative to polyoma form I DNA) indicative of the presence of polyoma dimers. The remainder had higher relative mobilities (0.68-0.7) and were shown by analysis of restriction enzyme digests to contain one unit each of pBR322 DNA and polyoma, with both possible orientations being represented. The same type of analysis showed that the hybrid with a relative mobility of 0.5 comprised one molecule of pBR322 and two of polyoma DNA linked head-to-tail, whereas the hybrid with a relative mobility of 0.52 consisted of one molecule of pBR322 linked to a head-to-tail dimer of polyoma DNA with a deletion of about 800 base pairs. None of 48 hybrids of pBR322 and polyoma DNA joined through their *Eco*RI sites contained more than one molecule of polyoma DNA, but two contained two pBR322 molecules and both orientations of polyoma DNA with respect to pBR322 were found. Figures 1 and 2 show the analyses of



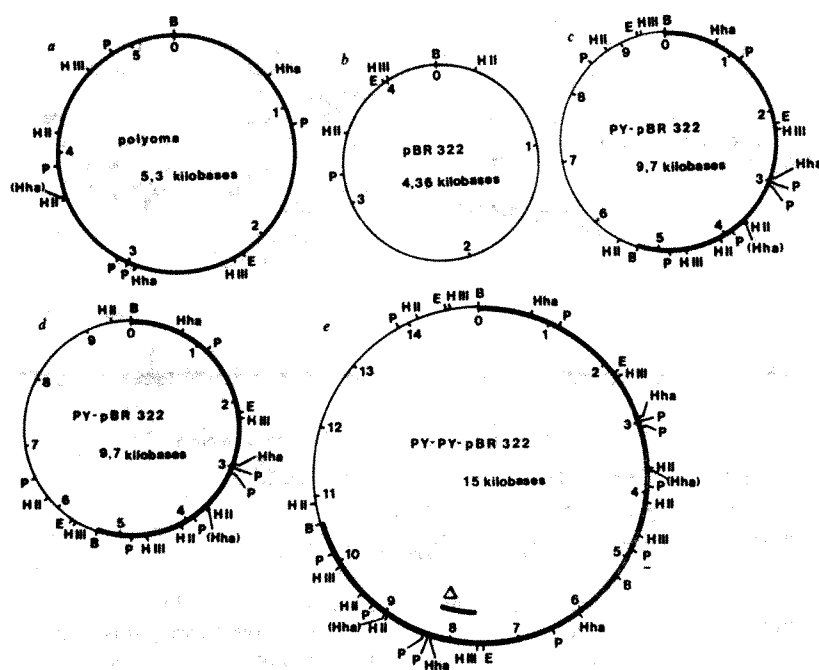


Fig. 1 Restriction maps of polyoma DNA, pBR322 DNA and some polyoma-pBR322 recombinant DNAs. *a*, Polyoma DNA (5,307 base pairs; from ref. 17). The DNA used here lacked the *HhaI* site (in parentheses) at position 26.4 from the *EcoRI* site. *b*, pBR322 DNA (4,361 base pairs; G. Sutcliffe and W. Gilbert, personal communication). *c*, Monomeric polyoma-pBR322 hybrid, joined at the *BamHI* sites so that the reading direction of the 'early' genes of polyoma and the *Tet* gene of pBR322 coincide (defined as head-to-tail). *EcoRI* cleavage should yield fragments of 7,075 and 2,575 base pairs. *d*, As *c*, except the reading directions are opposed (defined as head-to-head). *EcoRI* cleavage should yield fragments of 6,175 and 3,475 base pairs. *e*, Dimeric polyoma-pBR322 hybrid, joined at *BamHI* sites so that all units are head-to-tail, as defined above. Cleavage by *EcoRI* should yield fragments of 7,075, 5,300 and 2,575 base pairs. The other three possible combinations of orientations would yield the following *EcoRI* fragments: pBR322-Py (head-to-head)-Py (tail-to-head), 6,175, 5,300 and 3,475 base pairs; pBR322-Py (head-to-tail)-Py (head-to-head), 6,200, 6,175 and 2,575 base pairs; pBR322-Py (head-to-head)-Py (tail-to-tail), 7,075, 4,400 and 3,475 base pairs. B, *BamHI*; PstI; E, *EcoRI*; HII, *HindII*; Hh, *HhaI*.  $\Delta$ , site of deletion in hybrid D<sub>5</sub>.

some of these plasmids and their structures.

Polyoma DNA was also inserted into DNA from several bacteriophage  $\lambda$  derivatives, some of which could lysogenise their host, some which had temperature-sensitive repressors, and some which carried functional genes for phage-mediated recombination (*red*). Linear polyoma DNA, obtained by digestion with *EcoRI*, was ligated to the restricted vector DNA, and viable phage DNA molecules were recovered by *in vitro* packaging<sup>5</sup>. Recombinant phage were distinguished from parental ones on the basis of plaque morphology, or their inability to permit synthesis of  $\beta$ -galactosidase (as demonstrated by their failure to generate blue plaques on an appropriate plate) in their infected host<sup>6</sup>, or by plaque hybridisation with <sup>32</sup>P-labelled polyoma RNA<sup>7</sup>. Plaques of recombinants were picked and taken through at least two cycles of purification from individual plaques before preparation of stocks by lytic infection. DNA from purified phage was analysed by digestion with restriction enzymes and electrophoresis in agarose gels (Fig. 3), and by electron microscopy of heteroduplexes formed

with DNA from their parent vector, or another recombinant. All but one of the recombinants analysed contained a unit length insert of polyoma DNA and both orientations with respect to each vector were represented; the exception,  $\lambda$ -Py 48, contained less than a unit length of polyoma DNA, the deletion being located at one of the junctions between polyoma and vector. Some of the recombinants obtained are described in Fig. 4. Various approaches failed to yield any stable recombinants containing more than one molecule of polyoma DNA, although biological screening on the basis of restriction coefficient to *EcoRI* (ref. 8) and ability to plate on *pel*<sup>-</sup> (ref. 9) host strains indicated that several of the phage initially recovered contained dimers of polyoma DNA which were lost on further growth and purification. The reasons for this instability are unknown, but with the same vectors stable recombinants containing two molecules of pBR322 linked head-to-tail were obtained.

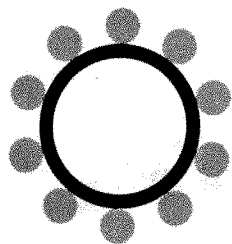
Recombinants were also made in  $\lambda$  derivatives with polyoma DNA cleaved at sites other than that for *EcoRI*. Partial digests of polyoma DNA with *HindIII* (for which it has two targets) were ligated with restricted DNA from two  $\lambda$  vectors<sup>6,22</sup> (Fig. 4)

Table 1 Infectivity of polyoma-pBR322 and polyoma- $\lambda$  hybrids

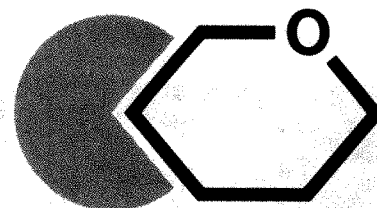
DNA preparation		Uncleaved DNA		Specific infectivity	
Prep. no.	Units of polyoma DNA per hybrid	PFU per $\mu$ g total DNA	PFU per molecule	PFU per $\mu$ g full-length polyoma DNA ( $\times 10^{-4}$ )	PFU per complete linear polyoma molecule ( $\times 10^7$ )*
<i>a</i> , Polyoma-pBR322 hybrids					
A <sub>2</sub>	1	<1	<10 <sup>-11</sup>	3.2	1.9
D <sub>6</sub>	1	<1	<10 <sup>-11</sup>	2.8	1.6
E <sub>8</sub>	1	<1	<10 <sup>-11</sup>	2.6	1.5
G <sub>3</sub>	1	<1	<10 <sup>-11</sup>	3.7	2.2
D <sub>3</sub>	2	7.5 $\times 10^4$	1.2 $\times 10^{-6}$	2.3	1.4
D <sub>5</sub>	1.9	8.7 $\times 10^3$	1.4 $\times 10^{-7}$	2.2	1.3
Py	—	2.2 $\times 10^6$	1.3 $\times 10^{-5}$	7.9	4.6
<i>b</i> , Polyoma- $\lambda$ hybrids					
$\lambda$ Py12	1	<1	<10 <sup>-10</sup>	1.6	0.94
$\lambda$ Py35	1	<1	<10 <sup>-10</sup>	2.6	1.5
$\lambda$ Py37	1	<1	<10 <sup>-10</sup>	1.8	1.1
$\lambda$ Py48	<1	<1	<10 <sup>-10</sup>	<10 <sup>-4</sup>	<6 $\times 10^{-5}$
$\lambda$ Py57	1	<1	<10 <sup>-10</sup>	2.3	1.4
$\lambda$ PyPy	—	9 $\times 10^5$	5.2 $\times 10^{-6}$	6.4	3.8

The DNA was cleaved to completion with *BamHI* endonuclease (*a*) or *EcoRI* (*b*) and the enzyme was inactivated by heating at 60 °C for 15 min. Appropriate dilutions of unrestricted and restricted DNA, respectively, were assayed on mouse embryo cells for plaque formation using the DEAE method<sup>10</sup>. Py, polyoma DNA.

\* The molecular weights (MW) used for the calculations are polyoma DNA, 3.51  $\times 10^6$ ; pBR322, 2.89  $\times 10^6$ ;  $\lambda$  DNA, 32  $\times 10^6$ .



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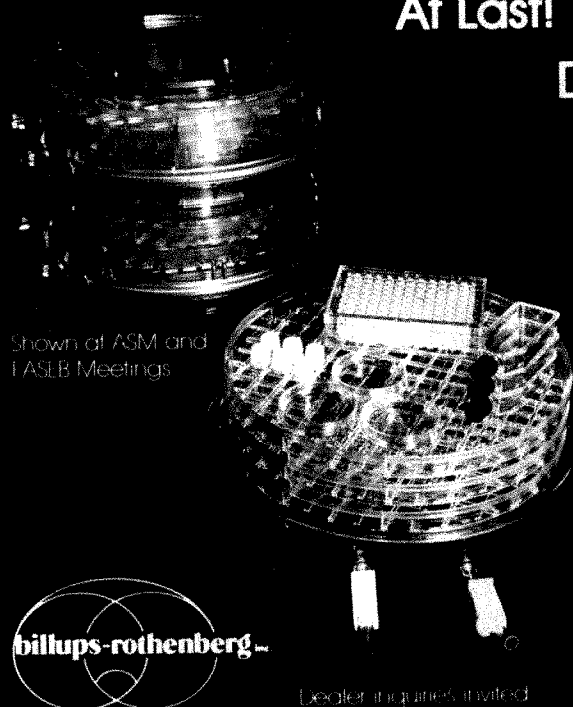
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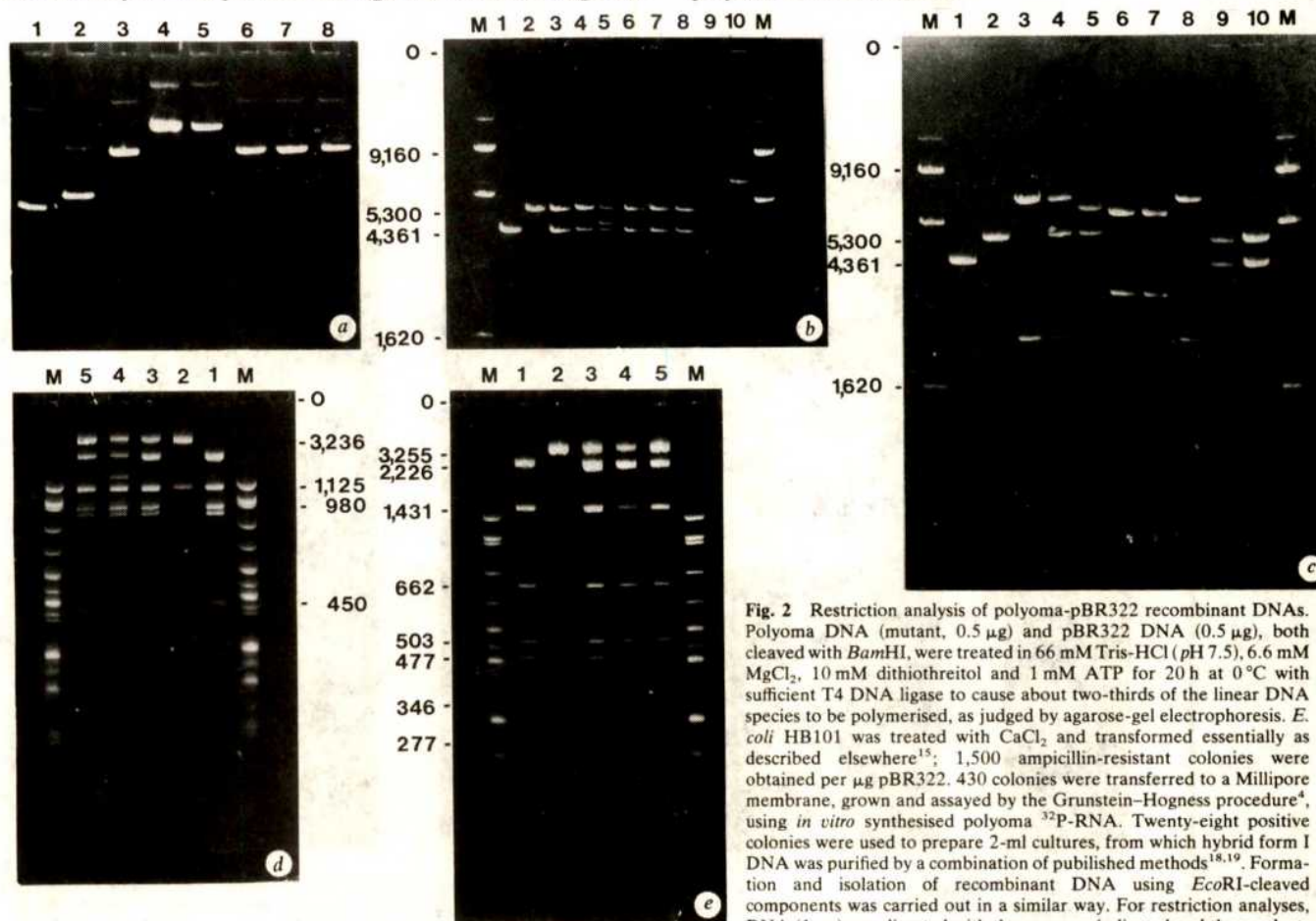


and again, two derivatives were used, one able to lysogenise its host and the other not. None of the recombinants isolated contained more than one molecule of polyoma DNA, but all contained the full monomer and with each vector three of the four possible derivatives were isolated (Fig. 4).

The infectivity of the polyoma-pBR322 hybrid DNAs before and after cleavage with the restriction enzyme used to generate the joined fragments was compared with that of polyoma virus DNA, using the DEAE method for transfection of mouse embryo cells<sup>10</sup>. Table 1 shows the specific infectivities of some of the polyoma-pBR322 hybrids joined through the *Bam*HI site. Hybrids containing one unit length of polyoma virus DNA gave less than 1 plaque-forming unit (PFU) per  $\mu$ g of hybrid DNA (less than 1 PFU per  $10^{11}$  DNA molecules), but cleavage of these hybrid DNAs with *Bam*HI resulted in a specific infectivity (in terms of unit lengths of polyoma DNA) similar to that of the viral DNA cleaved with *Bam*HI. Similar results were obtained with five of six hybrids that contained monomers of polyoma virus DNA joined to pBR322 through the *Eco*RI cleavage site

(results not shown). In no case was the uncleaved DNA infectious, but five of the hybrid DNAs were infectious after cleavage with *Eco*RI, which liberated a unit length linear viral genome. The reason for the lack of infectivity of the remaining hybrid after restriction remains uncertain; a non-infectious molecule may have been cloned, or infectivity of the polyoma DNA moiety may have been lost on passage of the hybrid in bacteria.

In contrast to the intact hybrids containing one unit of polyoma DNA, those containing 2 or 1.9 tandem head-to-tail genomes of polyoma virus DNA were infectious. The hybrid containing a dimer of polyoma virus DNA was 10% as infectious as intact form I viral DNA (in terms of PFUs per polyoma DNA molecule), whereas the hybrid containing 1.9 viral genomes was about 1% as infectious as viral DNA. The specific infectivity after cleavage with *Bam*HI of the two hybrids containing more than one unit of polyoma virus DNA was similar to that of similarly treated viral DNA in terms of PFUs per unit length of polyoma DNA molecules.



**Fig. 2** Restriction analysis of polyoma-pBR322 recombinant DNAs. Polyoma DNA (mutant, 0.5  $\mu$ g) and pBR322 DNA (0.5  $\mu$ g), both cleaved with *Bam*HI, were treated in 66 mM Tris-HCl (pH 7.5), 6.6 mM  $MgCl_2$ , 10 mM dithiothreitol and 1 mM ATP for 20 h at 0°C with sufficient T4 DNA ligase to cause about two-thirds of the linear DNA species to be polymerised, as judged by agarose-gel electrophoresis. *E. coli* HB101 was treated with  $CaCl_2$  and transformed essentially as described elsewhere<sup>15</sup>; 1,500 ampicillin-resistant colonies were obtained per  $\mu$ g pBR322. 430 colonies were transferred to a Millipore membrane, grown and assayed by the Grunstein-Hogness procedure<sup>4</sup>, using *in vitro* synthesised polyoma <sup>32</sup>P-RNA. Twenty-eight positive colonies were used to prepare 2-ml cultures, from which hybrid form I DNA was purified by a combination of published methods<sup>18,19</sup>. Formation and isolation of recombinant DNA using *Eco*RI-cleaved components was carried out in a similar way. For restriction analyses, DNA (1  $\mu$ g) was digested with the enzymes indicated and the products analysed by electrophoresis on agarose gels in the presence of ethidium bromide<sup>20</sup>. a, Native DNA samples: (1) pBR322 (the major band is form

I DNA, the minor bands are probably due to form II and to spontaneous pBR322 dimers, forms I and II). (2) Polyoma DNA; (3-8) hybrids linked through *Bam*HI sites: A<sub>2</sub>, D<sub>3</sub>, D<sub>5</sub>, D<sub>6</sub>, E<sub>8</sub>, G<sub>3</sub>. b, *Bam*HI cleavage. (1) pBR322; (2) polyoma; (3-8) hybrids linked through *Bam*HI sites: A<sub>2</sub>, D<sub>3</sub>, D<sub>5</sub>, D<sub>6</sub>, E<sub>8</sub>, G<sub>3</sub>; (9, 10) hybrids linked through *Eco*RI sites: A<sub>91</sub>, B<sub>46</sub>; M, marker:  $\phi$ 29 DNA incompletely digested with *Eco*RI. c, *Eco*RI cleavage. Lanes numbered as in b. d, Double digestion with *Pst*I and *Bam*HI. (1) Polyoma DNA; (2) pBR322; (3, 4) hybrids with more than one unit polyoma linked through *Bam*HI sites: D<sub>3</sub>, D<sub>5</sub>; (5) monomeric hybrid linked through *Eco*RI site: E<sub>8</sub>; M, marker: pCR1 DNA digested with *Bsp*II. e, Triple digestion with *Hind*II, *Hind*III and *Bam*HI. Lanes numbered as in d. One of the larger ('dimeric') hybrids (D<sub>3</sub>) gave two fragments on digestion with *Bam*HI: the longer one, which had the mobility of linear polyoma DNA, was present in about twice the molar yield (as estimated visually) of the shorter one, which had the mobility of linear pBR322. *Eco*RI cleavage of hybrid D<sub>3</sub> gave three products. The largest (7,100 base pairs) and the smallest (2,600 base pairs) of these had the same mobility as the *Eco*RI fragments of hybrids A<sub>2</sub> and G<sub>3</sub>, respectively, whereas the third fragment (5,300 base pairs) co-migrated with linear polyoma DNA. The hybrid D<sub>3</sub> therefore consists of one molecule of pBR322 and two of polyoma DNA linked head-to-tail, in the orientation shown in Fig. 1e. The other large (dimeric) hybrid D<sub>5</sub> gave three fragments on cleavage with *Bam*HI, one with the mobility of pBR322, one with the mobility of polyoma DNA, and one with a mobility corresponding to a chain length of about 4,700 base pairs. Thus, one of the polyoma DNA units has a deletion of about 600 base pairs. Digestion of D<sub>5</sub> with *Eco*RI yielded the same two smaller fragments (5,300 and 2,600 base pairs) as did the dimeric polyoma plasmid D<sub>3</sub>; however, the largest (7,100 base pair) fragment was replaced by a shorter one of about 6,400 base pairs (c). As shown in Fig. 1 legend, only the arrangement of Fig. 1e yields fragments of 5,300 and 2,600 base pairs; therefore, D<sub>5</sub> probably consists of one molecule of pBR322 linked to a head-to-tail polyoma dimer with a deletion of about 800 base pairs in the region yielding the large *Eco*RI fragment. The site of the deletion was located more precisely by analysing the products obtained after simultaneous digestion with *Bam*HI and *Pst*I on the one hand and with *Bam*HI, *Hind*II and *Hind*III on the other. The resulting fragments were compared with those from a similar digestion of polyoma DNA, pBR322 and the dimeric polyoma plasmid D<sub>3</sub>. As shown in d, hybrid D<sub>5</sub> doubly digested with *Bam*HI and *Pst*I, lacked a 1,850-base pair fragment specific to polyoma which was replaced by a shorter fragment of about 1,300 base pairs; after digestion with *Bam*HI, *Hind*II and *Hind*III one (of two) polyoma-specific fragments of 1,450 base pairs was replaced by one of ~700 base pairs. No pBR322-specific bands were missing. The deletion is thus located at the position indicated in Fig. 1e. The orientation of the A<sub>2</sub> and G<sub>3</sub> hybrids is indicated in Fig. 1c and the D<sub>6</sub> and E<sub>8</sub> hybrids in Fig. 1d.



Polyoma virus was isolated from several cell cultures that had been infected with the restricted plasmid containing monomeric polyoma DNA or with the unrestricted plasmid containing dimeric polyoma DNA; in all cases, the progeny had only the two *HhaI* sites characteristic of the mutant DNA used to produce the recombinant DNAs. To eliminate the possibility that free polyoma DNA (linear or circular) was present in the hybrid DNA preparations before transfection, 1- $\mu$ g samples of the unrestricted hybrids containing dimers of polyoma DNA were subjected to agarose-gel electrophoresis. The DNA was denatured and transferred from the gel to cellulose nitrate membranes<sup>11</sup> and polyoma-specific sequences were located by hybridisation with <sup>32</sup>P-labelled polyoma virus DNA followed by autoradiography. No radioactive band was detected in the positions of forms I or II polyoma DNA; 0.1 ng of polyoma DNA could be detected in parallel lanes. This experiment shows that the observed infectivity was due to the polyoma-pBR322 hybrid molecules and not to free viral genomes in the preparations, as a possible contamination could not exceed about 0.01%.

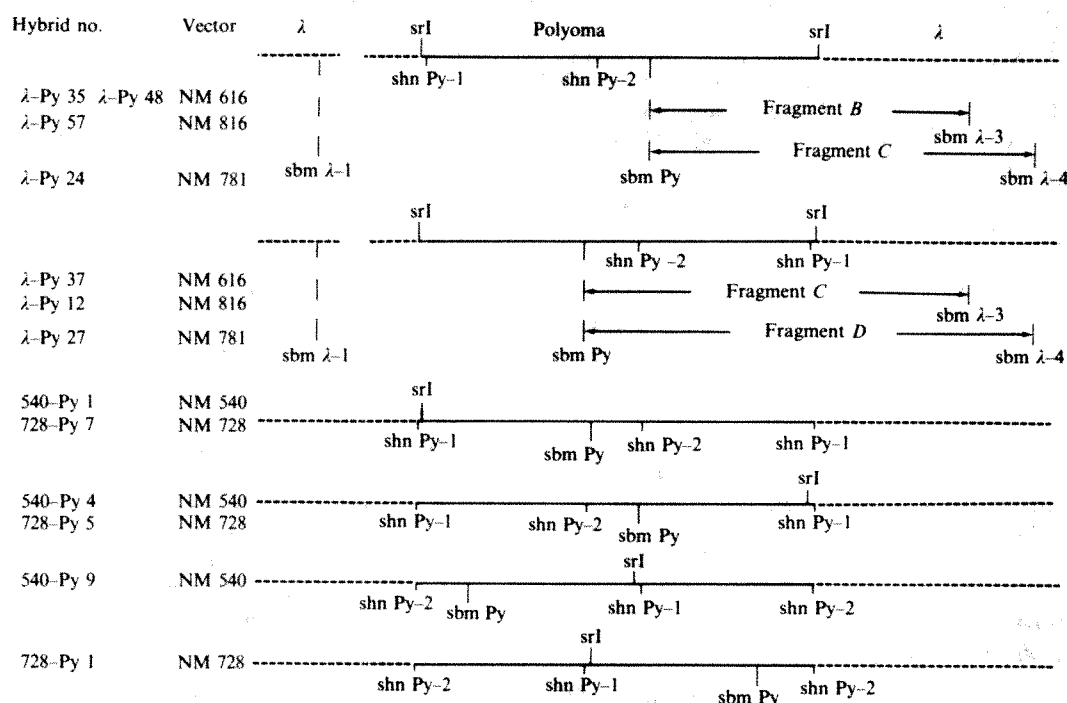
The results for the infectivity of the polyoma- $\lambda$  hybrids before and after restriction were essentially the same as those for the monomer polyoma-pBR322 hybrids. Less than 1 PFU per  $\mu$ g of hybrid DNA (<1 PFU per  $10^{10}$  DNA molecules) could be detected after infection of mouse cells with any of the uncleaved polyoma- $\lambda$  hybrids that contained a single copy of the polyoma virus genome, but cleavage with the appropriate restriction enzyme led to an infectivity similar to that of a molar equivalent of restricted polyoma DNA (Table 1). Of the polyoma- $\lambda$  hybrids constructed through *EcoRI* sites, seven of the eight different types of DNA molecule were infectious after digestion with *EcoRI*, but DNA from one hybrid ( $\lambda$ -Py 48), which was shown to have a deletion at one of the sites of attachment, was not infectious after such treatment. Similarly, the hybrids constructed through the *HindIII* sites, one of which is in the early and one in the late region, were not infectious as intact DNA molecules.

These experiments show that it is possible to prepare and maintain in *E. coli* strain HB101 plasmids containing two units



**Fig. 3** Electrophoresis of DNA from  $\lambda$ -Py hybrids in 1% agarose gels. The samples were digested with the following enzymes: tracks 1-7 and 13-17, *Bam*HI; tracks 8, 10, 11, 19, *Eco*RI; tracks 9, 18, *Bam*HI and *Eco*RI; track 12, *Hind*III and *Eco*RI. The samples in tracks 1, 9, 12 and 19 serve as reference for the size of fragments; the samples in tracks 11 and 12 show over-digestion with *Eco*RI, but nevertheless clearly show the release of unit length Py DNA from  $\lambda$ -Py 35 on digestion with *Eco*RI and provide the necessary reference points for known fragments of  $\lambda^+$  DNA in the digest with *Hind*III and *Eco*RI (refs 20 and 22). The hybrid phage were made as follows. The vector DNA (usually 1  $\mu$ g) was digested with the appropriate restriction enzyme and incubated with Py DNA (about 0.5  $\mu$ g digested with the same restriction enzyme) and T4 DNA ligase (0.1 units) in 66 mM Tris-HCl, pH 7.5, 10 mM MgCl<sub>2</sub>, 1 mM EDTA, 40 mM NaCl, 10 mM 2-mercaptoethanol and 0.1 mM ATP (0.1 ml) at 10 °C for 3-6 h and then 0 °C for at least 24 h and sometimes several days. In some experiments linear dimers of Py DNA were isolated by agarose-gel electrophoresis of samples of Py DNA (10  $\mu$ g) that had been digested with *Eco*RI, heated to inactivate the enzyme and then incubated with T4 ligase. The section of gel containing the linear dimers was dissolved in saturated potassium iodide solution<sup>21</sup> and the DNA adsorbed on hydroxyapatite from which it was recovered by elution with 0.4 M potassium phosphate, pH 7, and dialysed against 0.3 M NaCl and then 10 mM Tris-HCl, pH 8, and 1 mM EDTA. Linear Py DNA cleaved at one of the two *Hind*III targets was prepared by partial digestion in conditions previously shown to give no detectable fragments of Py DNA. Viable phage DNA molecules were recovered by transfection of competent cultures<sup>15</sup> of *E. coli* *r*<sub>k</sub><sup>-</sup> *m*<sub>k</sub><sup>-</sup> *recA* prepared as described by Lederberg and Cohen<sup>16</sup> or, more frequently, by *in vitro* packaging using cells that had been UV-irradiated<sup>5</sup>. Plaques screened as indicated in Fig. 4 were picked into phage buffer containing CHCl<sub>3</sub> and re-plated. After two such cycles, plate lysates were prepared and used to infect small-scale liquid cultures. From these lysates 1-l cultures were prepared by infection of cells at a m.o.i. of 1 followed by growth for 3 h with vigorous aeration. Lysis was completed by addition of CHCl<sub>3</sub> and phage recovered from the clarified lysates by precipitation with polyethylene glycol (Carbowax 6000, to 10% w/v, NaCl to 0.5 M) overnight at 4 °C. The precipitates were recovered by centrifugation, extracted with phage buffer and after clarification by centrifugation at 10,000 r.p.m. the phage were isolated by centrifugation on CsCl step gradients followed by equilibrium centrifugation in 41.5% w/w CsCl (ref. 14). Phage bands were collected and dialysed against 10 mM Tris-HCl, pH 8 and DNA prepared by phenol extraction followed by dialysis. Samples were digested with restriction enzymes as indicated above and analysed by electrophoresis in agarose gels (1% in 40 mM Tris-acetate, pH 8.2) which were photographed under UV light after staining with ethidium bromide (0.5  $\mu$ g ml<sup>-1</sup>). In some cases the gels were soaked in NaOH solutions to denature DNA fragments for transfer to cellulose nitrate membranes<sup>11</sup> and hybridisation with <sup>32</sup>P-labelled Py RNA. The fragments A, B, C and D all hybridised with the Py probe. The MW of these fragments deduced from their electrophoretic mobilities are: A,  $2.5 \times 10^6$ ; B,  $2.75 \times 10^6$ ; C,  $3.25 \times 10^6$ ; D,  $3.9 \times 10^6$ . Fragment E is characteristic of *Bam*HI digests of DNA from  $\lambda$  derivatives and is the left terminal fragment of the molecule. All operations from transfection or packaging of ligase reaction products to isolation and restriction digestion of the hybrid phage DNA were carried out in a Category III laboratory and all cultures and lysates were inoculated and handled in glove boxes, rather than laminar flow cabinets. Digested DNA was analysed in Category I laboratories.

**Fig. 4** Orientation of polyoma DNA within  $\lambda$  in the  $\lambda$ -Py hybrids. Only part of the  $\lambda$  genome is included in the diagrams showing the orientation of the polyoma DNA within  $\lambda$ . The vectors are described elsewhere<sup>6,22,23</sup>; NM 816 is a  $\lambda$ CI derivative of NM616. Targets for *EcoRI* are denoted by srl, and targets for *BamHI* and *HindIII* by sbm and shn, respectively. The orientations were based initially on the size of the fragments (determined from their electrophoretic mobility in 1% agarose gels) released by digestion with *BamHI*. In the case of the hybrids made through the *EcoRI* sites, the important fragments were A, B, C and D (Fig. 3), but fragment B ( $2.75 \times 10^6$  daltons) and fragment D ( $3.9 \times 10^6$  daltons) are the same size as fragments that would be released by the action of *BamHI* on head-to-head or tail-to-tail dimers of polyoma (2.8 or  $3.9 \times 10^6$  daltons). Heteroduplexes made between all the hybrid DNAs and their parent vectors showed, however, that all but one of the hybrids contained an inserted DNA fragment equal in length to polyoma DNA. The exception was  $\lambda$ -Py 48, which had a slightly smaller fragment inserted. The fragment in the *BamHI* digest of  $\lambda$ -Py 40 (Fig. 3, track 3) with the same mobility as polyoma DNA was, in fact, not polyoma DNA, but a fragment containing both  $\lambda$  and polyoma sequences; the orientation of the polyoma DNA in this hybrid is not shown here. Heteroduplexes between pairs of hybrids confirmed that the polyoma inserts in  $\lambda$ -Py 35,  $\lambda$ -Py 48 and  $\lambda$ -Py 57 were in the same orientation, but in the opposite orientation from those in  $\lambda$ -Py 37 and  $\lambda$ -Py 12; similarly, the orientation of the polyoma in  $\lambda$ -Py 24 was shown to be opposite to that in  $\lambda$ -Py 27. The heteroduplexes between  $\lambda$ -Py 35 and  $\lambda$ -Py 48 showed a deletion of about 0.7% of the total DNA located at 59% from one end of the molecule. This, together with the MW of fragment A in a *BamHI* digest ( $2.5 \times 10^6$ ), showed the presence of a deletion of about  $0.25 \times 10^6$  daltons (about 350 base pairs) in hybrid  $\lambda$ -Py 48 and digestion of the DNA with *EcoRI* showed that the deletion removed one of the *EcoRI* sites (srl-2) at which the polyoma and vector DNAs had been joined. Further digests of the hybrid DNA preparations with *HindIII* and digests with both *EcoRI* and *BamHI* (results not shown) confirmed these conclusions and assignments of orientation. The orientations of the polyoma DNA in the  $\lambda$ -Py hybrids made through *HindIII* sites and the determination of the *HindIII* site in the polyoma DNA at which the insertion had occurred were confirmed by an extensive analysis (by gel electrophoresis) of the digestion products formed with *BamHI*, *HindIII* and *EcoRI*, acting singly and in combination (results not shown).



of polyoma DNA linked head-to-tail at the *BamHI* site. The failure to recover hybrids that contain dimeric polyoma DNA linked to plasmid DNA or to phage  $\lambda$  DNA through the *EcoRI* site is not understood although the changes in characteristics observed during the propagation of some of the polyoma- $\lambda$  hybrids recovered by *in vitro* packaging suggest that phage genomes containing dimeric polyoma DNA were formed but were unstable. It seems that with both *red*<sup>+</sup> and *red*<sup>-</sup> phage in *rec*<sup>+</sup> or *recA* host cells the dimeric polyoma DNA is converted rapidly into monomer size by some recombinational events in the bacterial cell. None of the hybrid phage constructed through the *HindIII* site contained dimers of polyoma DNA. However, more recent results suggest that phage containing dimers of polyoma DNA can be made through the *BamHI* site although their stability is unknown.

The fact that cloned polyoma DNA, when excised at the site of joining, has an infectivity very similar to that of form III DNA derived from polyoma virions is evidence for the remarkable fidelity of the cloning procedure and the stability of a eukaryotic sequence replicated in a prokaryotic host. The finding that hybrid DNA containing only one unit length of polyoma DNA is not infectious to cells permissive for polyoma shows that intracellular excision of full-length, circular or linear polyoma DNA from the hybrid must be a very rare event. In contrast, excision of polyoma DNA from a hybrid containing a head-to-tail dimer is relatively efficient in mouse fibroblasts and probably results from legitimate recombination. A hybrid containing a head-to-tail dimer of polyoma DNA with an 800-base pair deletion in which, due to the location of the deletion (Fig. 1e), only 1.3 units of polyoma DNA are available for excision of a unit length viral DNA, had 10% of the infectivity of the plasmid containing the full dimer. This suggests that the frequency of

excision may be related to the target size for possible recombinational events. The production of SV40 virus from adenovirus-SV40 hybrids has also been observed to depend on the extent of duplicated SV40 sequences present<sup>24</sup>. The observed infectivity of hybrid DNA containing dimers of polyoma DNA resembles the results obtained by Jaenisch and Levine<sup>12</sup>, and Israel *et al.*<sup>13</sup>, who found that linear and circular oligomeric SV40 DNA was infectious to permissive cells and gave rise to SV40 particles containing monomeric DNA.

Experiments designed to test the transfer of recombinant DNA from *E. coli* to an animal cell or to an animal can only be useful if the recombinant DNA itself is potentially infectious. The demonstration that hybrid DNA containing head-to-tail dimeric polyoma DNA is highly infectious to mouse fibroblasts whereas that containing a monomeric DNA insert is not, justifies an extension of the experiment to *in vivo* studies. In these studies bacteria containing such hybrid plasmids and phage will be administered to cultured mouse cells and to mice which will then be scored for polyoma infection.

After completion of this work two reports were published on the cloning of polyoma virus DNA in the pBR322 plasmid and bacteriophage  $\lambda$  in the enfeebled *E. coli* host  $\chi$ 1776 (refs 25, 26). In one case a dimer of polyoma DNA was shown to have been cloned in  $\lambda$  but in an unstable fashion. Consistent with the results presented here that recombinants containing duplicated polyoma virus sequences are biologically active *in vitro*, these workers found that only recombinant DNA preparations containing dimer polyoma sequences were biologically active when injected into animals.

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## Cytoplasmic synthesis of plastid polypeptides may be controlled by plastid-synthesised RNA

It is generally agreed that some plastid polypeptides are coded by plastid DNA and the remainder by nuclear DNA, and that some plastid polypeptides are synthesised on plastid ribosomes and the remainder on cytoplasmic ribosomes<sup>1,2</sup>. Studies on two mutant lines of barley (*Hordeum vulgare* L. 'albostrians' (M4205) and 'Saskatoon') have helped the understanding of the integration of the plastid and nucleocytoplasmic systems. In both lines a nuclear mutation is expressed as an irreversible mutation of the plastid DNA yielding pigment-deficient plastids whose segregation leads to white-striped leaves and later to pure white leaves and shoots<sup>3,4</sup>. The white leaves do not contain

plastid ribosomes and no protein synthesis can be detected in the plastids, although cytoplasmic ribosomes are present in normal amounts<sup>5,6</sup>. Immunoelectrophoresis of extracts of white leaves has failed to detect either the plastid-synthesised large subunit or the cytoplasmically synthesised small subunit of ribulose-bisphosphate carboxylase/oxygenase, or coupling factor CF<sub>1</sub>, which has three plastid-synthesised and two cytoplasmically synthesised subunits, or the cytoplasmically synthesised ferredoxin-NADP<sup>+</sup> reductase<sup>6,7</sup>. We report here that two cytoplasmically synthesised plastid enzymes<sup>8</sup>, phosphoribulokinase (EC 2.7.1.19) and D-glyceraldehyde-3-phosphate: NADP<sup>+</sup> oxidoreductase (phosphorylating) (EC 1.2.1.13), occur in considerably reduced amounts in white leaves and deduce how the plastid may control the cytoplasmic synthesis of plastid polypeptides.

Enzyme activities were determined in the clear supernatants of homogenates made from barley leaves and were carried out for each enzyme both before and after *in vitro* activation, as described in Table 1 legend. Normally pigmented leaves of M4205 gave results for the two enzymes similar to those obtained previously for several other species, in that *in vitro* activation was substantial in extracts from etiolated leaves but proportionately smaller in extracts from illuminated green leaves because illumination of green leaves causes activation *in vivo*<sup>9</sup>. Table 1 shows that plastid pigment-deficient leaves contained measurable amounts of both enzymes and that the activities increased in response to *in vitro* activation. Thus the enzymes were present in both dark-grown leaves and in leaves subjected to 48 h of illumination, their activities falling within a range of 0.5-3.6% of those of the normally pigmented leaves of M4205. Absorption spectra and their second derivatives of intact white leaves have shown the presence of small amounts of carotenoids and chlorophyll at less than 1% of the plastid pigment content of normally pigmented leaves of M4205 (T.B. and A. Meister, unpublished). Parallel experiments with 'Saskatoon' barley yields results similar to those shown in Table 1 for M4205. Note that the *Euglena gracilis* mutant W<sub>3</sub>BUL, which lacks plastid DNA, also contains very low amounts of cytoplasmically synthesised plastid polypeptides<sup>10</sup>.

Plastid pigment-deficient leaves of both mutant barley strains seem to show slightly 'leaky' inhibition of the cytoplasmic formation of phosphoribulokinase and glyceraldehyde-phosphate dehydrogenase (NADP<sup>+</sup>). This contrasts with data for genetically normal seedlings grown at high non-permissive temperatures (28-34 °C) where the newly formed parts of the leaves are free from plastid ribosomes and deficient in plastid pigments<sup>11</sup>. The non-permissive temperature may function by preventing the assembly of plastid ribosomes<sup>12</sup>. Pigment-

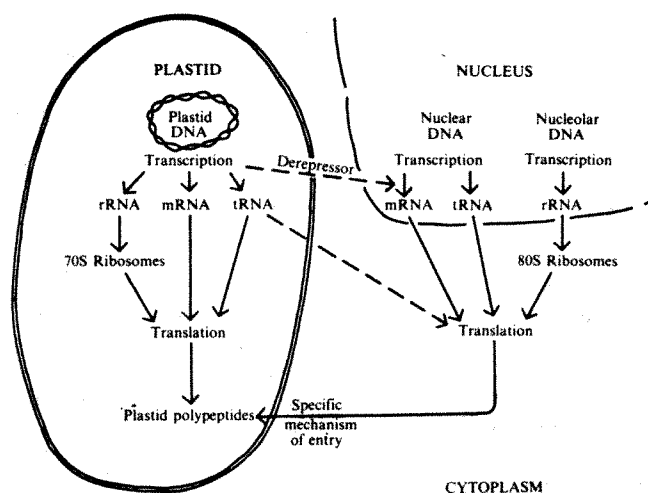


Fig. 1 Two alternative possibilities for the control of the cytoplasmic synthesis of plastid polypeptides by RNA transcribed from the plastid genome (effects expressed by broken lines).

**Table 1** Comparison of the phosphoribulokinase and glyceraldehyde-phosphate dehydrogenase (NADP<sup>+</sup>) activities of leaves of the barley mutant 'albostrians' (M4205) which either possess or are deficient in plastid pigments

	Enzyme activity (units per g fresh weight)			
	Phosphoribulokinase		Glyceraldehyde-phosphate dehydrogenase (NADP <sup>+</sup> )	
	Without <i>in vitro</i> activation	With <i>in vitro</i> activation	Without <i>in vitro</i> activation	With <i>in vitro</i> activation
7 d dark growth				
Plastid pigments normal	9.7	12.3	3.2	4.3
Plastid pigments deficient	0.15	0.37	0.09	0.15
7 d dark growth plus 2 d illumination				
Plastid pigments normal	49	51	14.1	16.6
Plastid pigments deficient	1.2	1.8	0.07	0.22

Two barley seedling first leaves were weighed and then homogenised in 5 ml of ice-cold extraction medium (100 mM Tris-HCl, pH 7.8 and 2 mM dithiothreitol) for 3 min in a Tenbroek homogeniser and the enzyme activities were determined in the supernatant obtained after 1 min centrifugation at 5,000g. Both enzymes were assayed spectrophotometrically from NAD(P)H consumption measured at 344 nm and 30 °C as previously described<sup>9</sup>. Phosphoribulokinase was activated by 12 min preincubation of 0.1 ml of extract with 50 µmol Tris-HCl, pH 7.8, 2 µmol dithiothreitol, 10 µmol MgCl<sub>2</sub>, 2 µmol ATP and 40 µmol KCl at 30 °C in a total volume of 0.38 ml. Glyceraldehyde-phosphate dehydrogenase (NADP<sup>+</sup>) was activated by 5 min preincubation of 0.1 ml extract with 50 µmol Tris-HCl, pH 7.8, 10 µmol MgCl<sub>2</sub>, 4.8 µmol EDTA, 3 µmol ATP and 5 µmol 3-phosphoglycerate at 30 °C in a total volume of 0.34 ml. Each value represents the mean from two separate leaf samples.

deficient sections of rye leaves grown at the non-permissive temperature (32 °C) contained normal amounts of the cytoplasmically synthesised photosynthetic carbon pathway enzymes, including phosphoribulokinase and NADP<sup>+</sup>-dependent glyceraldehyde-phosphate dehydrogenase, and furthermore these activities were found to accumulate within unpigmented plastids<sup>13</sup>. These pigment-deficient leaves also synthesised and accumulated the small subunit of ribulose-bisphosphate carboxylase/oxygenase in the absence of any synthesis of the large subunit<sup>14</sup>.

This work with seedlings grown at high temperatures established that the presence of functional chloroplasts is not necessary for the accumulation of cytoplasmically synthesised plastid stroma enzymes, and suggests that the failure of such enzymes to show appreciable accumulation in unpigmented leaves of the mutant barley was a result of the inhibition of their synthesis. As back-crossing experiments<sup>3</sup> have shown that the nuclear genome of the mutant barley is stable and as the inhibition of the cytoplasmic synthesis of plastid polypeptides was not found until the plastid DNA became defective, it may be deduced that the inhibition of the cytoplasmic synthesis of plastid polypeptides resulted from the loss of a product or products, arising from plastid DNA, which controlled this process. As plastid pigment-deficient leaves of the mutants and of the high temperature-grown seedlings all lacked ribosomes and did not show polypeptide synthesis in the plastids, whereas the former lacked and the latter showed the cytoplasmic synthesis of plastid polypeptides, it seems evident that such a product could not have been a plastid-synthesised polypeptide or the metabolic product of the enzymatic activity of such a polypeptide. The pigment-deficient plastids of the mutants had undergone an irreversible change in their DNA, whereas the DNA of the plastids of the heat-treated seedlings is assumed to have been unaffected, as the effects of the heat treatment were reversed by return to a permissive temperature<sup>15</sup>.

The most direct explanation for the inhibition of the cytoplasmic synthesis of plastid polypeptides in the pigment-deficient barley mutants is the assumption that they are deficient in RNA transcribed from the plastid DNA. This RNA may function in derepressing the transcription of nuclear DNA coding for cytoplasmically synthesised plastid polypeptides. Alternatively it may function as tRNA necessary for the cytoplasmic translation of mRNA coding for plastid polypeptides in agreement with the recent report that 7–9 plastid DNA cistrons of *Euglena gracilis* code for tRNA molecules which function with cytoplasmic polyribosomes and which are distinct from the

tRNA which functions in polypeptide synthesis in the plastid<sup>16</sup>. These mechanisms, shown in Fig. 1, may be considered in relation to other proposals that organelle gene products may control the nucleocytoplasmic synthesis of organellar polypeptides for mitochondria<sup>17</sup> and chloroplasts<sup>2</sup>.

Our deductions are not compatible with the 'cytoplasmic control principle' as formulated by Ellis<sup>1</sup> for the regulation of organellar protein synthesis and which states that "cytoplasmic products control organellar protein synthesis, but that the converse does not occur". We do not dispute that cytoplasmic products may control organellar protein synthesis but cannot accept the second part of this statement. On the contrary, we consider that there are also cases in which plastid products control the amount of synthesis of plastid proteins on cytoplasmic ribosomes.

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# matters arising

## Short-term storage and wind power availability

ANDERSON *ET AL.*<sup>1</sup> present a further analysis of Ryle's suggestion on the role of alternative energy sources<sup>2</sup> that wind power could be used in conjunction with 150-h thermal storage to provide domestic space heating. Ryle contended that "as soon as such storage is introduced a number of alternative sources of energy can be compared on an equal basis with a nuclear system"; in other words, that wind power can not only replace nuclear power as an energy producer but, when used in conjunction with 150-h storage, can contribute just as reliably to meeting peak electricity demands. Our disagreement with Ryle is on the latter point.

In our previous paper<sup>3</sup> and our comments on Diesendorf and Westcott<sup>4</sup>, we attempted to show that 150-h storage was inadequate for matching wind generator output to the heating load and as a result would have little, if any, effect in reducing the amount of firm generating capacity needed for meeting peak demands on the electricity supply system. We believe that the data presented by Anderson *et al.*<sup>1</sup> support this view.

Figure 1 of ref. 1 shows that over the 17-yr period considered, wind power in conjunction with 150-h storage would have failed to maintain room temperature to within 3 °C of the target temperature (20 °C) for 14% of the time, while Fig. 2 shows that during the period February–March 1975 the temperature would have fallen below 17 °C for 65% of the time. We contend that the occupants would resort to supplementary direct electrical heating during such periods with the result that the peak load on the supply system would be little different from what it would have been in the absence of wind generation. Consequently, unless storage of the type advocated by Ryle could be economically provided for periods much longer than 150 h, wind power would operate only as a fuel saver.

Anderson *et al.*<sup>1</sup> also suggest that the optimum ratio of rated to annual average wind speed for a wind turbine would be closer to a value of 1.5 than the 2.3 ratio used by ETSU in Energy Paper 21, which we also adopted for our estimates. We acknowledge that such a choice of ratio would reduce the time of zero output and hence the storage requirement. However,

an estimate we have made from an analysis of 16 yr of wind data from four of the sites of ref. 3 suggests that the reduction in energy output would be well above the estimate of 20% so far as the higher wind speed sites, including offshore locations, are concerned. For such sites a mean annual wind speed of 14 knots at the standard 10 m recording height would be more typical than the 12 knots on which calculations are based in ref. 1. We estimate that, in the former case, the energy loss would be closer to 40%, a reduction which would outweigh the savings on capital cost. We agree, however, that the economic optimum is likely to be somewhat lower than 2.3 even for very windy sites, the exact value being machine design dependent. Anderson *et al.* suggest that the energy deficit resulting from operation at a ratio of 1.5 can be regained by increasing the radius of the rotor. It would seem to us that having optimised the ratio of the rated to mean wind speed at a given site for a given design of machine, the size of rotor would be maximised within the constraints of engineering feasibility.

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ANDERSON *ET AL.* REPLY—Leicester *et al.* have for the most part accurately restated our quantitative results<sup>1</sup>. With reference to the need for a supplementary supply, we would stress that the system we assumed was (intentionally) the most stringent in that, even with perfect correlation between available wind energy and heating demand, the house temperatures would only just be maintained at 20 °C. Nevertheless, the lowest temperature reached over the entire 17-year period was 10 °C, at a time when the outside temperature was ~0 °C. The system was, therefore, always able to supply at least 50% of the energy required (heating demand being assumed proportional to the difference between internal and external temperatures). Even if the energy shortfall on this isolated occasion

had to be met entirely by the conventional grid, the electrical heating demand would be reduced by ~50% over that necessary in the absence of wind power, which we would not describe as 'hardly different'.

The question of turbine rating has apparently not been fully appreciated by Leicester *et al.* Our analysis (which was based on calculations involving actual wind data and not just estimates) depends only on the statistics of the wind distribution at a particular site and is otherwise completely independent of the precise value of mean wind speed. The loss in total annual energy output for a change of turbine rating from 2.3 V to 1.5 V is relatively independent of the particular site and corresponding mean wind speed as can be seen from Fig. 7 of the ETSU report<sup>2</sup>; this figure, which shows the results for seven sites, including one of the higher wind speed sites referred to by Leicester *et al.*, indicates values of this loss in the range 15–25%, rather than the 40% estimated by Leicester *et al.* The specific value of 12 knots used in our Table 2 (ref. 1) was adopted simply to allow actual figures for power output and so on to be quoted; the conclusions would have been similar for any reasonable value of adopted mean wind speed.

The optimisation of costs for a particular design is a complex problem and the engineering constraints involve not only the size of the rotor but also, for example, the limitations of gearbox torque and tower strength. The same annual energy output can be obtained by the use of a larger but more lightly-loaded rotor operating over a lower range of wind speeds, allowing the use of a smaller gearbox and alternator and reducing the structural loadings of the tower. On the basis of the relative costs given by ETSU<sup>2</sup> this will usually lead to a more economical design.

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TO CONCLUDE this correspondence the authors have been invited to provide a



final summary of the main points of agreement and disagreement between them.

THERE are two principal points of disagreement: (1) the extent to which wind power (with 150-h storage) can provide a reliable heating supply; and (2) the variation of net power output with aerogenerator rating.

(1) Ryle<sup>1</sup> considered the question of domestic heating demand. He concluded that wind power with the addition of 150-h storage on the consumers' premises could meet this demand at all times and thus replace an equal amount of conventional or nuclear generating plant.

In an analysis covering a six-month period, Leicester *et al.* (ref. 2 and above) found periods of a week or more during which the 150-h storage, initially full, would have been completely discharged and therefore unable to meet any of the heating demand. They pointed out that at such times there would have been little reduction in the heating demand imposed on the electricity grid and, consequently, little saving in the amount of thermal plant needed to meet peak demands.

Anderson *et al.* later proposed<sup>3</sup> a modified system with a store in which the heat output is a linear function of the energy remaining in the store. In this system as the heat output from the store fell short of that required the demand on the grid would progressively increase. They found that over a 17-year period this demand would never exceed about half the average heating load, with a corresponding reduction in the required installed thermal power station capacity. Thus, although a wind energy system in which there was no surplus capacity would be unable to supply the entire heating load, it could, nevertheless, make a significant power as well as energy contribution.

Leicester *et al.* agree that this second proposal is of interest, but further work will be required before the full implications of such a system can be assessed. (2) The disagreement on the second point is less easy to explain. Based on the analysis of wind data, the Cambridge group suggest that changing the rated speed of the aerogenerator from 2.3 to 1.5 times the mean site wind speed decreases the annual energy output by between 15 and 25% (in agreement with the data given in the ETSU paper) whereas the CEGB group, from a similar analysis, derive a figure closer to 40%. The two groups agree that the precise figure will be a function of the generator characteristics and the wind speed distribution function and this matter warrants further study.

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## On an environmental model for the type Kimmeridge Clay

TYSON *ET AL.*<sup>1</sup> have proposed that Kimmeridgian coccolithic limestones were the result rather than the cause<sup>2</sup> of extreme anaerobic conditions analogous to those in the Black Sea today. Anoxic water columns develop where a salinity or temperature gradient causes density stratification which restricts circulation. However, anoxic mid-water columns develop in some areas due to high productivity in the euphotic zone<sup>3</sup>. If circulation was restricted, perhaps by topography, this process could go to extreme, therefore the suggestion of Gallois<sup>2</sup> cannot be discounted. The microlaminated marls associated with oil shales may form at maximum development of anoxic conditions. However, observations of major developments of the coccolithic limestones contradict this proposition for their origin. Also, carbonate will tend to dissolve below the O<sub>2</sub>–H<sub>2</sub>S interface<sup>4</sup>.

The Rope Lake Head Stone Band interbedded with the oil shales is bioturbated with *Rhizocorallium* and encrusted with oysters. The White Band contains distinct burrows at certain horizons and ripple cross-lamination. The evidence is conclusive, the major developments of coccolithic limestone occurred in aerated bottom water. Transpositional structures within the limestones indicate the substrate was unstable and this would account for the lack of benthos in places.

I believe the oil shales mark the maximum stand of the O<sub>2</sub>–H<sub>2</sub>S interface. Vertical movement of this interface could cause the lithological change clay–bituminous shale–oil shale, but dilution by terrigenous material can account for it equally well. In the Kimmeridge Clay both factors interact. The coccolith limestones accumulated when circulation increased. The previously anoxic water would supply concentrated nutrients, in particular HCO<sub>3</sub><sup>-</sup>, and favour the propagation of coccoliths. This model accounts for incomplete cycles, such as, bituminous shale–coccoliths–bituminous shale and is more consistent with observational evidence.

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TYSON REPLIES—Irwin has proposed an interesting modification of the stratified water column interpretation for the cyclic sedimentation observed in the type Kimmeridge Clay. While we apparently

agree that the sequence clay–bituminous shale–oil shale represents a transition from aerobic to anaerobic bottom conditions (coupled with the progressive development of an O<sub>2</sub>–H<sub>2</sub>S interface in the lower part of the water column) our respective interpretations for the depositional conditions of the coccolith limestones are directly opposed.

On the basis of field observations Irwin claims that the coccolith limestones were deposited in aerobic bottom conditions when the dispersal of anoxic, nutrient-rich bottom water had promoted increased propagation of coccoliths. As I have only just completed a detailed examination of the sequence I must contradict Irwin's evidence: (1) The Rope Lake Head limestone, although bioturbated, is not oyster encrusted (and is not a true coccolith limestone). (2) Only a single poorly developed horizon of bioturbation occurs in the White Stone Band coccolith limestone, reflecting what was clearly a transient improvement in bottom oxygenation (for example, associated with limited advection due to a density current). (3) The White Stone Band coccolith limestone does not contain any evidence of bottom currents, but penecontemporaneous deformation structures do sometimes resemble ripples. (4) The coccolith limestones are devoid of benthos.

The remainder of Irwin's argument contains several inconsistencies. While solution of inorganic carbonates (such as, 'seekreides'<sup>1</sup>) is an important process below the O<sub>2</sub>–H<sub>2</sub>S interface, it is not relevant to this discussion for coccoliths are at present day accumulating on the floor of the Black Sea<sup>2</sup> (despite pH values of 7.6 ref. 3). If the deposition of coccolith limestones were initiated by the dispersal of anoxic bottom water (coincidental with the development of aerobic bottom conditions) then: (1) There is no reason why 'varve-like' microlaminations should form. (2) Any microlaminations would have been destroyed by bioturbation (there is no *a priori* reason to suppose the substrate was unsuitable for benthos—especially when one considers the bioturbation in the White Stone Band). (3) This would contradict the geochemical<sup>4</sup> and palynofacies evidence (ref. 5 and personal observations). (4) It would imply that the coccolith limestones should always be underlain by oil shales (that is, sediments representing anoxic bottom conditions) which they are not. (5) According to Gallois<sup>6</sup> the decay of the resultant phytoplankton bloom would recreate anoxic bottom condition anyway. (6) A greater degree of lateral variation would be expected. Bottom water dispersal events are recorded in the stratigraphic record, but not by laminated coccolith limestones<sup>7</sup>.

Any belief in a stratification/O<sub>2</sub>–H<sub>2</sub>S interface model is incompatible with Gallois' original hypothesis<sup>8</sup> which was an alternative to the restricted basin models.

If one does believe in stratification one should accept that with the onset of stagnation the  $O_2$ - $H_2S$  interface will inevitably rise to the base of the euphotic zone (where it will participate in the plankton dynamics of the basin) unless one of several external influences disrupts the system<sup>7</sup>. Much more convincing evidence is required if Irwin seeks to replace a model which has the weight of Quaternary-Recent modern analogues behind it.

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## Calcium activation of the cortical reaction in sea urchin eggs

USING sea urchin (*Echinus esculentus*) eggs broken by shock discharge, or isolated egg cortices, Baker and Whitaker<sup>1</sup> found that about  $1 \mu M$  Ca was sufficient to activate exocytosis of half the cortical vesicles. They represent this figure as an order of magnitude lower than the figure we reported<sup>2</sup> (between 9 and  $18 \mu M$ ) using *Lytechinus pictus* sea urchin isolated cortices.

However, there is no discrepancy between our results. We measured the level of calcium required for nearly complete (>95%) exocytosis, and we have recently obtained a more precise estimate of  $12 \mu M$ , determined by breaking the eggs open in the test solutions. From Baker and Whitaker's Fig. 2, they observe 95% exocytosis at a Ca concentration of  $6 \mu M$  ( $pCa = 5.2$ ). Moreover, Baker and Whitaker derive an apparent stability constant<sup>3</sup> from a Ca-EGTA binding constant<sup>4</sup> ( $pK$ ) of 11. However, in physiological media a lower binding constant is appropriate<sup>5</sup>. We assumed a  $pK$  of 10.7 in our calculations for our HEPES-buffered solutions, as this value was reported<sup>6</sup> for similar phosphate-buffered solutions. This difference in binding constant accounts for the twofold difference remaining between our calculated Ca threshold for the cortical reaction and that offered by Baker and Whitaker.

The problem of selecting the appropriate binding constant for EGTA is a general one in studies of the role of calcium. We recently attempted to estimate the primary Ca-EGTA binding constant ourselves, using calcium-specific electrodes filled with Ca-DOPP mixed with

DOPP-n<sup>7</sup>. We could only obtain accurate measurements of calcium when the apparent binding constant of EGTA for Ca was reduced in PIPES-buffered low pH (6.5) and the monovalent cation concentration was kept to 0.13 M. We measured an apparent binding constant,  $pK' = 5.3$ , corresponding to a primary Ca-EGTA binding constant of  $pK = 10.6$ . Since high pH<sup>8</sup> and high ionic strength<sup>9</sup> are both reported to reduce the primary Ca-EGTA binding constant (but see ref. 10), it seems that even our estimate for the Ca threshold of the cortical reaction ( $12 \mu M$ ) is too low.

Baker and Whitaker also suggest that ATP is needed to maintain the cortical intracellular Ca store. We found that sperm, the Ca-transporting membrane ionophore A23187, and the parthenogenetic agent urea all release Ca from the same store<sup>11</sup>. Quantitative estimates of the amount of Ca released<sup>2,12</sup> compared to the cortical reaction threshold, suggest that the store is released into a small fraction of the cytoplasm at the cortex. As the store is replenished and can be discharged again about 45 min after release (when the cortical vesicles are gone), it is unlikely to be associated with the cortical vesicles themselves. The calcium store may reside in a subsurface endoplasmic reticulum, but this has not yet been observed in the appropriate cortical region. The calcium may be bound directly to the membrane instead.

We thank Sherwin Lee for help with the Ca-sensitive electrodes.

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**BAKER AND WHITAKER REPLY**—We are delighted that Zucker and Steinhardt find no serious disagreement between their results and ours.

It is unfortunate that one cannot be sure of the value of the free calcium concentration in physiological solutions buffered with EGTA to within half an order of

magnitude. As Steinhardt *et al.* pointed out<sup>1</sup> the most one can do is to specify total calcium, magnesium and EGTA concentration so that, should reliable affinity constants become available, the free calcium data may be recalculated. Although monovalent cation concentrations were not identical, a rather direct comparison of the results obtained on British and American urchins is possible because the calcium buffers used in our experiments<sup>2</sup> were made up in the same proportions of calcium, magnesium and EGTA as those specified in the study of the cortices of *Lytechinus*.

Much of the apparent discrepancy stems from Zucker and Steinhardt's somewhat peculiar definition of 'threshold' for cortical granule release as the calcium concentration giving release of 95% of the granules. Although the release of individual granules is an all-or-none process, our observations suggest that in a population of granules the rate of discharge is a smooth function of calcium concentration. Physiologically, it is likely that the abrupt onset of the cortical reaction can be attributed to the release of large amounts of calcium from a store<sup>3</sup>. In the presence of 5 mM ATP, a calcium test solution<sup>2</sup> which according to Steinhardt *et al.*<sup>1</sup> contains  $1 \mu M$  free calcium, initiates a cortical response which spreads as we have described and has discharged all the cortical granules within 2 min. At higher calcium concentrations the rate of discharge of the granules (expressed as a percentage of the total number) increases. A calcium activation curve obtained by scoring the proportion of granules remaining at 30 s has a similar half point to the data (Fig. 2a of ref. 2) for eggs subjected to high voltage discharge in solutions of different calcium concentration. Again, using the constant of Steinhardt *et al.*<sup>1</sup> the half activation point is about  $3 \mu M$ , although its position will depend on the time after addition of calcium at which the reaction is assessed. At its fastest, the discharge of cortical granules over the whole cortical fragment takes about half a minute. This is not dissimilar to the rate *in vivo*<sup>4</sup>. Without information as to the time of observation, the half point of activation in the cortices of *Lytechinus* cannot strictly be estimated, but might at its lowest be  $6 \mu M$ . At worst, as it is not clear whether concentrations of calcium less than  $12 \mu M$  cause any granule discharge in *Lytechinus*, there remains an order of magnitude difference between the two species in the lowest calcium concentration which has been demonstrated to produce a cortical response.

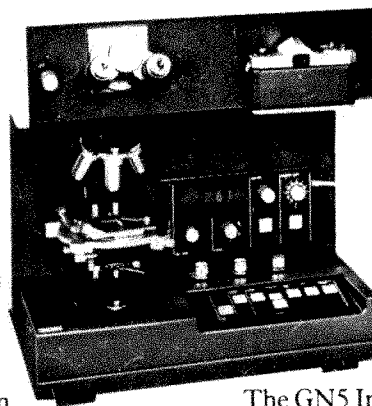
Although this difference in the affinity of the cortical reaction for calcium in the eggs of the British and American urchins may well have its explanation in species differences, or the uncertainties in free calcium concentration, our observations as to the critical importance of ATP in maintaining the properties of the

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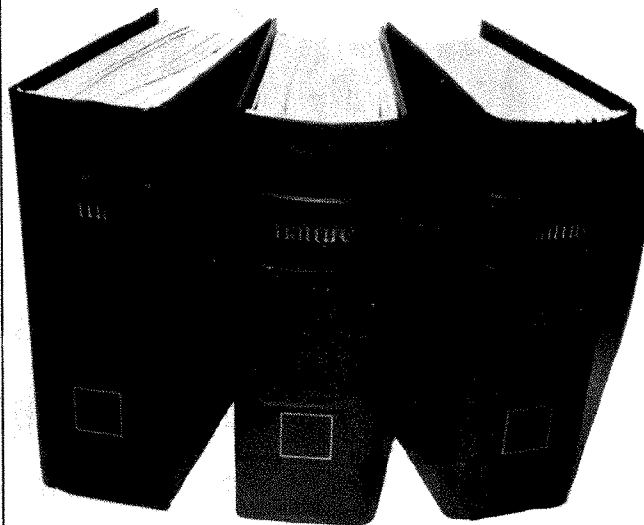
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physiological reaction<sup>2</sup> suggest a less trivial explanation. As experiments with *Lytechinus* were performed in the absence of added ATP, the decrease in calcium affinity which results from removal of ATP in *Echinus* may well be the factor responsible for the apparent differences in calcium affinity in the two species.

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### Fusion or lysis of vesicles by $\text{Ca}^{2+}$ ?

GINSBERG showed<sup>1</sup> that sonicated phosphatidylserine (PS) vesicles in the presence of large concentrations of  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  did not retain sucrose and he concluded that the final structures had lost the form of closed vesicles. As such, he proposes the cation effect to be one of lysis and the  $\text{Ca}^{2+}$ -PS system to be an inappropriate one for the study of membrane fusion. It is not surprising that the final product of the PS-metal interaction cannot retain solutes such as sucrose, as the vesicles collapse and internal volume is lost<sup>2</sup>. The PS membrane repeat determined by X-ray diffraction is 53 Å in 2 mM  $\text{Ca}^{2+}$ ; 67 Å in 10 mM  $\text{Mg}^{2+}$  (ref. 2) and 71 Å in 1 M  $\text{Na}^+$  (ref. 3). The term 'membrane fusion' refers to the formation of larger membranous structures by contact and mixing of the parent membranes. This is in contrast to the mixing of membrane lipids by diffusion of the components, as proposed for the dimyristoyl lecithin-dipalmitoyl lecithin system<sup>4</sup>. Recent experiments<sup>3</sup> have shown that release of contents is nearly second order in vesicle concentration and is concomitant to aggregation, demonstrating vesicle contact in leakage experiments. That  $\text{Ca}^{2+}$  induces fusion, that is, mixing of membrane components, is indicated by the formation of large cochleate structures, which on addition of EDTA, become huge vesicles capable of entrapping large molecules<sup>5</sup>.  $\text{Mg}^{2+}$  alone is less effective<sup>6</sup> but in its presence, only small concentrations of  $\text{Ca}^{2+}$  are required for obtaining larger structures<sup>2,3</sup>. Similarly, in the dimyristoyl phosphatidylglycerol-dipalmitoyl phosphatidylglycerol<sup>7</sup> and in phosphatidic acid-phosphatidylcholine vesicles<sup>8</sup>,  $\text{Ca}^{2+}$  induces mixing of the lipids, that is, fusion. Our view is that fusion requires a destabilisation of the membranes in contact until the joint membrane is arranged. Model systems are intended to be approximations to the *in vivo* systems. A definition of

membrane fusion as a process without leakage is too restrictive and so far no experiment has ruled out leakage in fusion events. In model systems better approximating the *in vivo* system, such as mixed PA/PC vesicles<sup>8</sup>, mixing and retention of contents has been demonstrated. The interactions postulated to occur between the  $\text{Ca}^{2+}$  and PS in the pure system are still relevant to the mixed systems.

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### GINSBERG AND GINGELL REPLY—

The comments of Nir and Pangborn stem from an inadequate definition of membrane fusion. They claim that fusion is equivalent to the mixing of components from two contacting parent membranes. However, physiological membrane fusion apparently involves the transfer of aqueous contents from one membrane-bounded compartment to another without spillage into inappropriate spaces, as seen in phagosome-lysosome interaction and secretion. Any artificial system should fulfil this additional criterion to be biologically relevant<sup>1,2</sup>. Thus, the X-ray data cited by these authors<sup>3</sup> seem to invalidate their PS system as a paradigm for membrane fusion:  $\text{Ca}^{2+}$  converts PS into a multilayer containing no removable water. Such collapsed multilayers could result from the lysis of closed membranous forms by the mechanism we suggest<sup>1</sup>. The experiments described by Nir and Pangborn where loss of contents is reported to accompany vesicle aggregation may also be explained in terms of lysis:  $\text{Ca}^{2+}$ -induced vesicle rupture with loss of contents may provide the antecedent for immediate aggregation of the resultant membrane fragments.

Although  $\text{Ca}^{2+}$  is strongly implicated in biological membrane fusion<sup>4</sup>, there is no compelling reason to suppose that its action on dispersions of single acidic phospholipids resembles its interaction with mixed lipid membranes nor with the biomembrane systems in which fusion was first studied<sup>5</sup>. This point is underlined by the far greater  $\text{Ca}^{2+}$  sensitivity of natural vesicle fusion<sup>6</sup>. Gershfeld has recently shown<sup>7</sup> that sonicated vesicles are in a metastable state at temperatures exceed-

ing the lipid phase transition temperature. Thus, addition of divalent cations to PS vesicle suspensions may merely trigger a return to equilibrium by a variety of unknown pathways.

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### Some real communities are unstable

THE mathematical stability analyses of randomly constructed food webs of Pimm and Lawton<sup>1,2</sup> have emphasised the destabilising influence of omnivory. Their examination of a number of real food webs<sup>3</sup> seems to support their hypothesis that webs with many omnivores should be rare except in insect host-parasitoid systems. However, I analysed the real food webs cited as corroborative by Pimm and Lawton, that is, those of Askew<sup>4</sup>, Force<sup>5</sup> and Richards<sup>6</sup>, and found that none meet the criterion for Lyapunov stability. The validity of the models and their inherent assumptions appears questionable.

All webs were analysed using the observed signs of interaction and Pimm and Lawton's constraints on the selection of random magnitudes for parasitoid-host and herbivore-plant interactions. Signs for variable interaction, species A and B each serving as prey or predator for the other, were arbitrarily assigned. To investigate whether the occurrence of self-limited species influenced stability, runs were repeated with self-regulation terms removed (all principal diagonal elements equal to zero). Finally, self-limitation was introduced at the lowest trophic level (plant), a criterion used by Pimm and Lawton in constructing their random webs. Analysis of Richard's web was performed twice, once with the parasitoid-host and again with vertebrate predator-prey constraints used for the predatory arthropods. In no case were any of 50 runs for either the original or adjusted webs stable.

I included only primary interactions in abstracting a matrix from Force's web. The excluded terms (dashed lines in Force's paper<sup>5</sup>) represent secondary interactions, such as the rejection of a potential host which has been previously

parasitised by another parasitoid. The existence of this exploitative competition, which has been reported for many parasitoids<sup>4,7</sup>, implies that higher order interactions are common in parasitoid communities. However, higher order terms violate an assumption of the stability analysis.

If Lyapunov stability is a relevant feature of communities and extinction of some member(s) of each community is not imminent, there are several explanations for the observed discrepancy. Perhaps stable solutions exist, but have not been discerned, as randomly selected parameter values do not exhaust all possible combinations<sup>8</sup>, and the use of unequal constraints for different trophic levels prohibits tests of qualitative instability. Natural selection may drive a community towards a restricted region of parameter space where mathematical stability is realised<sup>9</sup>.

An alternative explanation is that the community models are inadequate. Coincident with Pimm and Lawton's predictions, the instability of these webs is apparently the result of high connectance (percentage of non-zero elements in the interaction matrix), reflecting the presence of many omnivores. In fact, both of Askew's webs include omnivores whose alternate prey occupy widely separated trophic levels. This is the most destabilising form of omnivory according to Pimm and Lawton. If these webs are in fact stable, perhaps the constraints on selection of interaction magnitudes are biologically unrealistic, as the other stability parameters (connectance, number of species) are observed quantities. It has been demonstrated that as the range of constraints is narrowed, implying lower interaction intensities, the probability of a random community exhibiting stability increases. The host switching of many parasitoids and the concomitant unequal use of alternate hosts suggests that a narrower range of constraints should be used for some of the parasitoid-host interactions.

The problems associated with the community models may involve more than just the determination of actual interaction intensities and their linearity. Two assumptions underly the models and the resultant stability analyses. First, communities are considered to be definable closed systems uninfluenced by immigration and chance extinction. However, real communities are dynamic entities. The delineation of ecologically meaningful boundaries of a community can be critical to stability analysis as some unstable communities are comprised of several stable subunits<sup>10</sup>. Furthermore, Levin<sup>11</sup> has demonstrated that immigration and spatial heterogeneity can profoundly influence community stability. The second assumption is that the interactions we are attempting to estimate, competition or predation, are responsible

for both the population dynamics of the species comprising the community and its organisation. Many predators, particularly parasitoids, can greatly influence the abundance of their prey as evidenced by successful implementation of biological control, but their role in community organisation is less clear. Similarly, the role of competition in structuring communities and controlling species abundances is a hotly contested issue<sup>12,13</sup>. Therefore, in light of the current lack of understanding of the determinants of community organisation, I question the predictive value of community modelling and mathematical stability analyses.

I thank Daniel Simberloff, Stan Faeth and Donald Strong for their comments.

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#### LAWTON AND PIMM REPLY—

Auerbach has overzealously applied an extremely simple model; Lotka–Volterra equations do no more than capture the shadow of real biological interactions. We used such models to generate qualitative predictions<sup>1</sup>, and deem it extremely unwise to attempt more quantitative fits to real webs. However, this does not mean that our hypotheses cannot be tested.

Our main qualitative prediction was that omnivory has a destabilising influence on food webs, the more so when predators are bigger and rarer than their prey, and have a large *per capita* effect on the things they eat. We were deliberately cautious in assessing this prediction, noting simply that it was 'encouraging' to find very complex webs in the real world in exactly those situations predicted by the models, that is amongst insect host–parasitoid interactions. More detailed analyses confirm that insect food webs do have significantly more omnivory than other webs<sup>2</sup>. Indeed none of several interesting qualitative predictions yielded by simple Lotka–Volterra models<sup>3,4</sup> are refuted by data from real food webs<sup>2</sup>. To test our hypotheses further, we would prefer to see whether these qualitative predictions still hold when the major assumptions of the models are changed, rather than try to force unmodified Lotka–Volterra equations beyond their sensible limits. For example, if incorporating spatial heterogeneity reverses our predictions (making

omnivory easier to achieve in non-parasitoid than parasitoid webs), then our results are a nonsense. We doubt whether any of the refinements listed by Auerbach will have this effect.

Far from refuting our models, Auerbach's analysis confirms our conclusion that omnivory is destabilising: it is much easier to find stable solutions for simple webs than it is for complex ones. At present, we see no evidence for claiming that the majority of persistent natural populations are best described by model analogues that are inherently unstable<sup>5,6</sup>. However, the detail needed to stabilise the models depends on the nature and rigour of the questions being asked. Auerbach suggests several possible reasons why his Lotka–Volterra models of real food webs are always unstable. Undoubtedly higher order interactions<sup>7,8</sup> (which do not violate the assumptions of a local stability analysis, but make non-equilibrium behaviour difficult to predict), the precise choice of parameter values (we put webs into the correct relative ranking: the numbers themselves were guessed, not measured, and may well have been wrong in detail?) and spatial heterogeneity are all important. The effects of spatial heterogeneity, in particular, must be incorporated before models can accurately predict observed levels of host depression by parasitoids<sup>9</sup>. (For a wide range of models of varying levels of complexity, yielding insights with different degrees of rigour, see ref. 10.)

However, Auerbach's note is valuable in drawing attention to biological details that must be important in determining the fine structure of food webs. Viewing webs as static entities with fixed links is no more than a crude beginning<sup>11,12</sup>. The concept<sup>13</sup> of webs 'resonating' between different configurations, each of low complexity, may well provide a valuable theoretical starting point for more sophisticated analyses.

We thank Mike Hassell and Simon Rallison for comments.

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# reviews

## Basis for an ethical system

Stuart Sutherland

*Beast and Man: The Roots of Human Nature.* By Mary Midgley. Pp. 377. (Harvester Press: Brighton, Sussex, 1979.) £7.50

*Beast and Man* has been widely praised by the litterateurs who contribute to the book pages of the surviving intellectual weeklies. It is a long, rather rambling book, in which it is difficult to discern a clear connected argument. Its main aim seems to be to establish a secure basis for ethics. Mary Midgley argues that, like other animals, man has many inborn drives: these drives can only come to fruition within the context of a culture. She argues that what is good for man must depend upon the nature of his motives: moral reasoning consists of the attempt to harmonise conflicting emotions and to give priority to those which are most deeply ingrained in our nature. It will be a comfort to scientists that she believes the scientific study of human nature can assist in making ethical decisions by revealing more about man's true nature. A second theme running through the book is that we are part of the Animal Kingdom and of the Universe and that one principle of morality is that man should have respect for other animate beings and indeed for the inanimate universe.

In preparing the ground for her own thesis, she assails previous views of the nature of goodness, but she frequently seems to espouse the mistakes of which she accuses others. For example, she attacks Aristotle for arguing that the ultimate human value must be the development of that function which is unique to man and not shared by other animals. She rightly remarks that "it must be shown separately that this differentia is itself the best human quality, that it is the point where humanity is excellent as well as exceptional". Yet later in the book she writes: "Should we therefore say that everything we want is good? In a minimal sense this is right...but...we must go further...because of a competition amongst our various wants. What is good in a stronger, more considered sense must be wanted not just by someone's casual impulse, but by *him as a whole*" [her italics]. The meaning of "him as a whole" is obscure, but supposing

it were to turn out — as it might — that hatred for outgroups was an inborn human characteristic, she would seem to be committed to the proposition that it was thereby good. Edward Wilson has emphasised that the mechanism of evolution depends on the selection of genes that survive and she criticises him for erecting the survival of the individual gene into a principle of morality, but she herself seems to be committing the same fallacy in arguing that whatever is an essential part of human nature must be good.

When she wishes to recommend a given course of action she tells her readers that it is "natural". She believes that: "Respect for other forms of life is certainly a natural feeling. It is not a mere inclination, it is a feeling that we must not destroy certain things — and one that is not isolated, but forms part of our central system of standards". She provides no arguments to show that this feeling is any more natural than delight in wanton cruelty; nor does she provide criteria for deciding that something is natural nor even attempt any clear definition of what the word means. The fact that something forms part of the "central standards" of some people cannot automatically make it good: many societies have as part of their central system of standards a belief in the duty to avenge themselves and their families for slights.

Much of her book seems to be based almost on word play. For example, she insists that the word "rational" is used not merely to describe the behaviour of someone who makes the correct moves in solving intellectual problems, but also the behaviour of someone who acts consistently from a well harmonised set of emotions. Her point about the use of language is right, but it does not seem to advance her argument and it is not clear that it is any more difficult to harmonise a set of bad motives than a set of good ones.

She frequently embarks on arguments that look promising, but they tend to peter out. For example, she uses a cunning analogy to attack one of Wilson's arguments: he believes that understanding how ethical systems have evolved and how our reasoning about morality is controlled by the nervous system will throw light on the nature of ethics. She points out that

discovering how the workings of the brain enable us to do mathematics will not illuminate the nature of mathematics: to understand that we must understand the relationship between numbers, how mathematical proofs work, and the standards by which to judge the correctness of a mathematical calculation or proof. But she makes no attempt to explain what the standards are that are used in moral argument or decision making. It is easier to agree about the canons of mathematics than to resolve a disagreement about ultimate goals.

Although the book as a whole is both unsatisfactory and unsatisfying it does contain nuggets of robust common sense, many of which are well put even if they are not entirely new. For example, in arguing for the continuity of man and the Animal Kingdom, Mary Midgley points out that we often project our own evil on to animals and use a double standard in evaluating their behaviour and our own. It is unreasonable to despise the fox who kills hens for sport more than those who ride to hounds. Again, she points out that we cannot abnegate from instilling a culture and norms in our children — an adult who tries to adopt a non-committal position and tells the child he need not accept a particular ideal until he is old enough to judge for himself is in effect saying "I do not take this seriously and nor need you" and is likely to instil norms of "timidity, shiftiness, and dilettantism". She proposes a sensible if slightly vague resolution of Wilson's dilemma over how altruism towards unrelated members of the same species may have originated: it may have been built on filial and parental instincts, and indeed affectionate gestures between adults mimic those made by parents to children and children to parents.

In view of her obvious sincerity and high-mindedness and the importance of the endeavour, it is a pity Mary Midgley has not produced a more cogent and carefully argued book, but most previous attempts to establish a secure basis for an ethical system have proved equally unsuccessful.

Stuart Sutherland is Professor of Experimental Psychology at the University of Sussex, Brighton, UK.



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## Protein denaturation

*Physicochemical Aspects of Protein Denaturation.* By S. Lapanje. Pp 331. (Wiley: Chichester, UK, and New York, 1978.) £19.50.

THERE has been no significant, full-length review of protein denaturation since 1970. Although many fundamental principles had been laid at the time, there has been a steady advance in the detailed understanding of denaturation in the intervening years. Further, the biological and industrial significance of conformational change and stability is more widely appreciated today, and more chemists, biochemists and physicists are ready to devote time and energy to rigorous studies of the dynamics of protein structure. The field of protein denaturation has a distinguished history, starting with Ramsden's observation in 1902 that "a dead frog placed in saturated urea solution becomes translucent and falls to pieces in a few hours". Anfinsen's thermodynamic hypothesis of folding, the concept of limited pathways, quantification of protein stability, the hydrophobic interaction and prediction of tertiary structure all owe something to denaturation studies, and many of these areas are still under active study.

This monograph sets out to survey the physicochemical basis of denaturation and therefore leaves the reader to apply the fruits of this approach to his own field of work and interest. Methods appropriate for studying denaturation are described; in each case a self-contained account of the fundamentals is given, with examples taken from conformational studies on proteins and polypeptides and references to recent reviews for further detail. Techniques include hydrodynamic, optical, light scattering, NMR and hydrogen exchange, although calorimetry, surprisingly, considering the author's interest, is given only passing mention.

The largest section of the book comprises a survey of experimental results obtained with a range of perturbants including heat, pH change, urea and guanidine, inorganic salts, organic solvents, and detergents. This draws together a wealth of detail from the literature and enables the reader to compare the different and frequently rather specific effects of these modes of denaturation. The results are well illustrated with data from original papers. It is useful to have the literature presented objectively and without the constraint of a particular theoretical viewpoint.

The chapter on the thermodynamics of denaturation, an aspect to which the author has made significant contributions, contains a good discussion of the two-state hypothesis and an up to date account of calorimetric studies and of the thermodynamics of thermal denaturation.

Although it is mentioned in several places that the denatured states may differ depending on the mode of perturbation, I could find no discussion of the dependence of derived thermodynamic parameters on the definition of the denatured state. Also, the values of  $n$  in the relationship  $K^1_D = Ac^n$  surely range wider than 12 to 20 — probably a misprint. The interpretation of biphasic kinetics of unfolding and refolding of proteins in terms of the proline isomerisation model is a particularly hot issue at the present moment and the short chapter on kinetics of denaturation provides a useful background to the various ways in which such experiments have been treated. This leads into a final chapter dealing more with interpretation of kinetic and thermodynamic experiments, particularly with respect to the mechanism of folding of globular proteins and the nature of the interactions of denaturants with proteins. As regards the latter, the author seems to favour the direct binding of urea molecules to a protein as a driving force in denaturation, and it is an interesting exercise to try and fit an alternative explanation to the results he quotes purely in terms of change in water structure.

This monograph is valuable as an unusually well organised survey of a massive literature. I found it stimulating, not because of strongly argued personal ideas, but because of the creative apposition of the different experimental approaches and interpretations which have been used in this field. It is, however, encouraging to find that his thermodynamic hackles are capable of being roused when his basic convictions are challenged (see p295). This review is valuable also in that the author emphasises the complexity of deriving and assigning thermodynamic parameters from systems as complex as proteins in multicomponent solvents.

The book is well produced and well illustrated. The index contains the reviewer's favourite enzyme but omits "two state transition". One is enormously impressed by the feat of writing (over 300 pages) in what is, to the author, his third language. Only occasionally is there a misplaced and, very occasionally, a misleading nuance. Most of these are so obvious that in more happy and responsible days one might have expected the publisher to have brought them to the author's attention. The duplication of equation 2-26 and the confusing layout of Table 4.4 also fall into this category. Nevertheless, this is a monograph that will be an essential reference work for all those concerned with the stability and dynamics of proteins and provides excellent value for money.

Roger H. Pain

Roger H. Pain is Professor of Physical Biochemistry at the University of Newcastle upon Tyne, UK.

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## Semiconductors textbook

*Semiconductors*. Second edition. By R. A. Smith. Pp. 523. (Cambridge University Press: Cambridge, New York and Melbourne, 1978.) Hardback £27.50; paperback £8.95.

THE publication of a second impression of any major textbook provides an indication of its value: its publication twenty years after the original edition in a field that had been developing very rapidly over most of this period gives further proof of the lasting value of the treatment which has not dated significantly. When R. A. Smith's book appeared in 1959 it provided the most detailed text on the then new and rapidly developing subject of semiconductor physics, with special emphasis on transport and optical properties. Now that the subject has reached what might be called mature age, the new edition is to be welcomed as a continuation of this tradition, with some significant modifications in order to introduce new topics and to update the bibliography. With the avowed intention of keeping the total volume constant, this was not an easy task, and the author may be congratulated on having, on the whole, succeeded rather well. As one part of the adopted strategy the reader is increasingly referred to the other major text by the same author, *Wave Mechanics of Crystalline Solids* (Chapman and Hall: London, 1961) and this means that the student will find the use of two textbooks more imperative than with the original edition.

The layout of material departs significantly from the original, partly to accommodate the additional subjects and partly to streamline the treatment. The first four chapters on elementary properties, energy levels, impurities and carrier concentration remain virtually unchanged. The original very extensive chapter on electron transport has been altered in several respects, one of which is the omission of the Boltzmann equation as the basis for the treatment of transport theory—in this reviewer's opinion, a retrograde step at the level aimed at in the book. Scattering mechanisms and high field effects are relegated to separate chapters and a new section is introduced on conduction at very low temperatures. Optical and high-frequency effects have been significantly expanded, the behaviour in high electric fields has been coupled with that in high magnetic fields to take account of recent advances, including the important transferred electron phenomena and carrier freezeout.

The original chapter dealing with the determination of semiconductor properties has been removed; and the chapters describing the properties of elemental and compound semiconductors have been shortened into one, no doubt on the reasonable basis that the volume of information on properties of semiconductors is so vast that it requires reference to specialist texts for any but the most elementary data. Instead, we have a short chapter on band structure and the effective mass approximation describing some recent developments such as the LCAO and the k.p methods and the pseudopotential. Another new chapter on "Some special topics" deals with excitonic molecules, electron-hole droplets, polarons and polaritons, tunneling in heavily doped materials, and the tunnelling spectroscopy and laser action in semiconductors, among others. The book closes with another new chapter on amorphous semiconductors providing a brief outline of this topic on a mainly descriptive basis.

The bibliography has been brought up to date in some of the more important respects but there is no pretence of giving complete listings which would become rapidly dated.

The overall impression is a very favourable one: this is still one of the most authoritative and comprehensive texts on this subject in one volume,

with a very detailed treatment of transport and optical properties and with a less detailed discussion of the band structures and of other physical properties of semiconductors. There is no significant discussion of any semiconductor devices, again on the reasonable assumption that this would make the text either much too long or too superficial.

One criticism of the presentation is the relatively inefficient use of diagrams: the information content of many of these is low for their size and some are too closely similar to represent real value—for example, Figures 13.3 and 13.4 giving the temperature dependence of the forbidden gap in germanium and in silicon. More economy in this respect would have permitted the inclusion of a wider range of information.

These are, however, minor criticisms of an otherwise excellent text which will continue to provide the basis of both undergraduate and postgraduate lecture courses and will represent a useful general reference text. The price, especially of the paperback edition, is very reasonable and the presentation is excellent throughout.

A. K. Jonscher

A. K. Jonscher is Professor of Solid-State Electronics at Chelsea College, University of London, UK.

## Standard reference work on the plastids

*The Plastids: Their Chemistry, Structure, Growth and Inheritance*. Second edition. By J. T. O. Kirk and R. A. E. Tilney-Bassett. Pp. 960. (Elsevier/North Holland/Biomedical: Amsterdam, New York and Oxford, 1978.) Dfl337; \$149.75.

THE first edition of *The Plastids* has become something of a standard reference book. Because of the great advances which have taken place since this first edition was published, it was already beginning, after only a decade, to show its age. The authors have therefore undertaken a complete revision of their work, and the new enlarged edition contains a vast amount of additional information not earlier available. Each chapter now begins with a useful summary which allows the reader to find his way quickly and easily through the book.

The present volume attempts to cover the whole plastid literature, and

therefore necessarily goes over much of the same ground as previously. The text is first class and references to new work are included as appropriate. The figures and diagrams contain much new material clearly set out. But I was greatly disappointed by the re-use of many old electron micrographs; in view of the rapid advances which have taken place in electron microscopical studies of plastids since 1968 I suspect that many of the authors credited with the pictures used would have welcomed the chance to offer new micrographs of material treated by more modern methods of fixation. The quality of printing of the micrographs, in spite of the glossy paper used and in view of the remarkably high price of the book, is generally poor and indistinct and many of the pictures lack the contrast which the originals undoubtedly possessed. The pictures carried forward from the first edition seem to have suffered most in this respect.

Part I, *Chemistry, Structure and Function of Plastids*, is greatly enlarged and now comprises chapters 1–12 (pp.1–250), an increase of 160 pages. This part of the book will be of great value to someone wishing to have a

good general summary of the properties of chloroplast proteins, lipids and pigments and an account of the fine structure of the different plastid types. There are also excellent short chapters on chloroplast permeability and photosynthesis. Dr Kirk is to be congratulated on the excellence of the summary of the new biochemical material and the development of ideas which he presents in this section.

Part II discusses the "Inheritance and Genetic Behaviour of Plastids" and is written by Dr Tilney-Bassett (chapters 13–22, pp.251–521). The form of presentation has obviously been completely rethought. New material has been incorporated, some old material discarded and a major rearrangement has been carried out. The new data on plastid mutagens and the causes of mosaic variegation are particularly valuable and the problem of sorting out of plastids is examined well. The origin and stability of chimaeras is discussed adequately, although the description demands a prior knowledge of the ontogeny of the shoot apex. The sections dealing with the reasons for the stability of periclinal chimaeras and for the existence of mericlinal chimaeras are acceptable but are sufficiently important to warrant a more extended treatment in the context of a discussion of plastid behaviour in higher plants. However, an expert who already knows the sort of details he is looking for is very likely to find them in this as in other sections, and the comprehensive subject, taxa and author indexes, will guide him well.

Chapter 22, which concludes Part II, is a completely new one which describes plastid inheritance in *Chlamydomonas*. It is a particularly helpful chapter and brings into focus the insight which the use of a unicellular plant can give to critical studies on extra-nuclear genes. As *Chlamydomonas* can be grown in accurately controlled conditions, it can be conveniently subjected to precise experimental manipulations; this is of particular importance in studying the expression of such genes.

Part III, written by Dr Kirk, examines the "Biochemical Basis for Plastid Autonomy and Plastid Growth" (chapters 23–28, pp.525–862). While keeping to the same general form of the first edition, it includes a mass of new information. For example, the section on biosynthetic capabilities now runs to 100 pages against a previous 30, an indication of an area of most rapid development in the past decade. Throughout Part III great care has been taken to ensure a good balance in the presentation. The whole topic of chlorophyll synthesis has now been assembled in one unit in the new volume, as opposed to the scattered treatment it received in the first edition, and de-

tails of modern work on the synthesis of  $\delta$ -aminolaevulinic acid and its conversion to protochlorophyll are included. In the past decade much information on nucleic acids and protein synthesis has been obtained and this is well discussed in chapter 26. The section on plastid development (chapters 27 and 28) has been expertly updated.

Part IV (chapter 29, pp.875–894) gives us the benefit of the authors' joint views of the directions in which future research may be expected to proceed. The coverage of references generally is good up to 1976, but the immense work of printing such a large volume means that the literature between 1976 and 1978 (date of publication) has of necessity had to be largely ignored. It is therefore satisfying to note that the literature already contains papers on several of the topics outlined jointly by the authors in

Part IV.

This edition of *The Plastids* is more readable than the earlier edition, but it is definitely not for beginners. However, workers in this very wide field will find an immense store of information and references to the literature. For such a large book there are remarkably few printing errors and grammatical inexactitudes. The efforts of revising *The Plastids* has been well worthwhile; the volume is very impressive. So too, unfortunately, is the price, which will put it beyond the grasp of all but the most dedicated of research workers. It will, however, find a proper place on library shelves in departments of biochemistry and plant sciences throughout the world.

F. R. Whatley

F. R. Whatley is Sherardian Professor of Botany at the University of Oxford, UK.

## Techniques at low temperature

*Experimental Techniques in Low-Temperature Physics*. Third edition. By G. K. White. Pp. 331. (Clarendon, Oxford University Press: Oxford 1979.) £15.

GENERATIONS of graduate students (including the present reviewer) were brought up on earlier editions of Guy White's book, which for years remained the only available supplement to the unwritten body of lore handed down by one's predecessors and supervisor and colleagues. It is a detailed exposition of the how of experimental physics at low temperatures: how to attain the low temperature, and how to measure it when you have got it; not only how to set about designing cryogenic apparatus, but also how to translate the design into a functioning and (one hoped) leakless reality. The third edition has been reworked more thoroughly than its predecessor, and is the better for it. Some of the less used sections have been pruned to make way for new material reflecting the growing emphasis on work below 1 K, and the whole book has generally been brought up to date.

The eleven chapters deal in succession with the production and storage of liquefied gases, heat exchangers, thermometry, heat transfer, temperature control, cryostat design, cooling with  $^3\text{He}$ , adiabatic demagnetisation, vacuum techniques, and with the physical properties of solids at low temperatures. There are substantial appendices giving tabular data on: vapour

pressures of cryogenic liquids; platinum resistance and thermoelectric thermometry; relevant physical properties of elements at room temperature; electrical resistivity; and on the thermal contraction and thermal conductivity of typical constructional materials. There is an extensive (and international) list of suppliers of the cryogenic equipment, components and materials referred to in the text.

White's book no longer stands alone, of course; there are also, for example, the admirable little text *Low Temperature Laboratory Techniques* by A. C. Rose-Innes (English Universities Press: London, 1973), as well as the excellent but more specialised works by O. V. Lounasmaa (*Experimental Principles and Methods Below 1 K*; Academic: London, 1974) and by D. S. Betts (*Refrigeration and Thermometry Below One Kelvin*; Sussex University Press: Brighton, UK, 1976); but it probably remains unique in the amount of useful practical detail which is included, and in covering so wide a sweep of low temperature physics and engineering.

It is a pleasure to welcome the third edition of *Experimental Techniques in Low Temperature Physics*, and to commend it to a new generation of research workers. It is readable, it is packed with ideas and information culled from laboratories all over the world, and it has an extensive and up-to-date bibliography. It is a book which every designer or user of low temperature apparatus will wish to have at his elbow.

P. V. E. McClintock

P. V. E. McClintock is Senior Lecturer in the Department of Physics at the University of Lancaster, UK.

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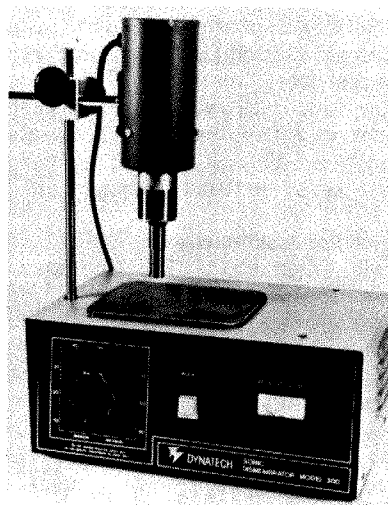
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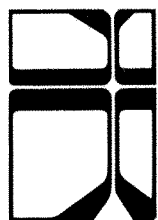
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2344(A)

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2288(A)

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2309(A)

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EUROPEAN TRAINING  
PROGRAMME  
in  
BRAIN AND BEHAVIOUR  
RESEARCH  
TRAINEESHIPS**

Traineeships of three, six or rarely nine months are offered to promising young scientists working in the area of brain and behaviour research in order to broaden their skills and knowledge in a field other than but related to their own. The grants are given to allow them to learn a particular technique abroad for which training is not offered in their own country. The trainee is expected to return to her/his original post upon termination of her/his training so that her/his institute will in turn be able to benefit from newly acquired skills.

All applications have to be accompanied by a letter of recommendation by the sending institute and by a letter of acceptance from the receiving institute. Preference is given to post-docs under the age of 35.

**TRAVEL GRANTS**

Travel grants are awarded to allow young research workers to visit another laboratory in connection with an on-going research project or to participate in international meetings and symposia. In addition special grants are to be awarded to students to enable them to take part in the yearly meetings of the European Neuroscience Association (ENA) which this year will take place in Rome on September 10-14.

Applications should be accompanied by letters of recommendation from the sending institute and of invitation from the institute to be visited. Applicants should be postgraduate research workers with the exception of applications to attend the yearly ENA meeting. In this particular case preference is given to postgraduate students.

The deadline for completed applications is *March 15 and September 15*. Further information and application forms may be obtained from:

Dr Stephanie Zobrist  
European Science Foundation  
European Training Programme in  
Brain and Behaviour Research  
1, quai Lezay Marnésia  
F-67000 STRASBOURG  
FRANCE  
Tel: (88) 35 30 63  
Telex: 890440

W187(A)

# Lancashire

County Council

COLLEGE OF AGRICULTURE

SCIENCE DEPARTMENT  
APPOINTMENT OF LECTURER



Applications are invited for the post of lecturer in the Department of Sciences. The duties will involve lecturing in Agricultural Chemistry, Animal Nutrition and related topics to a range of certificate and diploma courses as well as assisting in soil science and nutrition laboratory classes.

Candidates preferably should have an appropriate degree qualification or be completing such a course this Summer.

Salary to be within the Burnham Scales for staffs of Farm Institutes Grade 1A £3,192 to £5,334 (under review), the starting point dependent on approved qualifications and experience.

Further details and application forms, which should be returned within 14 days of the appearance of this advertisement, may be obtained from the Principal Agricultural Officer, Lancashire College of Agriculture, Myerscough Hall, Bilsborrow, Preston, PR3 0RY. Telephone: BROCK (0995) 40611

2286(A)

**IMPERIAL CANCER RESEARCH  
FUND**

**TECHNICIAN/  
RESEARCH OFFICER**

(1) required to assist in our Membrane Physiology Laboratory on projects involving the regulation of cellular metabolism and growth. Experience of cell culture techniques, column chromatography and gel electrophoresis desirable. Two years experience in a biochemistry laboratory essential. Reference MP1.

(2) to work in the Tumour Virus Genetics Laboratory on projects involving the analysis of nucleic acids of mammalian cells and viruses and the molecular cloning of eucaryotic genes. Experience in the preparation and standardisation of restriction enzymes, cell culture techniques, nucleic acid purification and gel electrophoresis would be desirable. Reference TVG1.

HNC or Degree in biological science, but school-leavers with two science 'A' levels may be considered. Salary range £3,600 to £5,250. For further information and application form write or telephone, quoting the above reference, to Miss S. M. Hurley, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2 on 242 0200 ext. 305.

2340(A)

**UNIVERSITY OF  
NEWCASTLE  
UPON TYNE**

**DEMONSTRATOR IN THE  
DEPARTMENT OF PSYCHOLOGY  
Applications are invited for a post of  
DEMONSTRATOR**

in the Department of Psychology tenable for a period of three years. Applicants should have a good honours degree in psychology, and preferably a postgraduate qualification. Some knowledge of computing would be an advantage. The person appointed would be expected to organise and share in the running of practical classes and also to undertake some tutorial teaching.

Salary will be at an appropriate point on the Grade 1B (Bar) scale £3,775 to £5,488 per annum operative from 1st October 1979, according to age, qualifications and experience. Membership of the appropriate University superannuation scheme will be required.

Applications, giving full details of age, qualifications and experience, together with the names and addresses of two referees, should be sent to the Head of the Department of Psychology, The University, Newcastle upon Tyne NE1 7RU not later than 13th July 1979. Please quote reference N.

2356(A)

**UNIVERSITY OF BRADFORD**

**2 Year FIXED TERM  
LECTURESHIP  
in Physics**

Applications are invited from suitably qualified physicists for the above post commencing October 1979. The research interests of the School of Physics lie in Physical Electronics and Nuclear Structure Physics; applicants should be qualified to pursue research in one of these fields. Consideration given to applicants completing a PhD this summer. Salary within the range £4,232 to £8,452 per annum (under review). Further details and application forms (to be returned by 13 July 1979) obtainable from the Registrar, Post Ref. PY/L/28/X, University of Bradford BD7 1DP.

2300(A)

**UNIVERSITY OF  
MANCHESTER**

**DEPARTMENT OF CHEMISTRY AND  
ARTIFICIAL KIDNEY UNIT,  
Withington Hospital  
RESEARCH FELLOW**

Applications invited from postdoctoral analytical chemists with a strong interest in medical research for a joint medical/chemical research programme on trace metal uptake through artificial kidney machines. The work will involve chemical analysis of aqueous and clinical samples for trace metals, by flameless A.A.S., and fundamental research into metal ion transfer across dialysis membranes; it will demand a high level of scientific ability. Experience of similar work is desirable. Starting salary in the range of £4,333 to £5,199 p.a. Superannuation. Contract period 2 years, starting as soon as possible. Further details from Dr. J. P. Day, Department of Chemistry, University of Manchester M13 9PL (phone 061-273 7121 ext 5374), to whom applications with full curriculum vitae should be sent as soon as possible.

2310(A)

**THE MIDDLESEX  
HOSPITAL MEDICAL  
SCHOOL**

(University of London)

Department of Biology as Applied to  
Medicine

A Research Assistant is required to work on the development of the mouse mammary gland with reference to invasiveness. The appointment would be for two years starting from October 1, 1979. While experience in cell biology would be an advantage, applications from recent graduates would be welcomed. Salary according to age and experience, up to £5,304 p.a. including London allowance. Applications, together with the names of two referees to Professor L. Wolpert, Department of Biology as Applied to Medicine, The Middlesex Hospital Medical School, London W1P 6DB.

2323(A)

The research organisation of Synthelabo, an Internationally recognized European Pharmaceutical Group located in Paris requires a

# SENIOR TOXICOLOGIST

To lead a team responsible for various projects within a developing toxicology group in the department of Biology.

The high success rate and rapid growth of this pharmaceutical group is related to the quality of people they employ and their continued commitment to research and development.

Applicants can be either male or female and should be a PH.D with at least 5 years post-doctoral experience in toxicology and preferably with industrial experience as well. Suitably qualified veterinarians and pharmacists with relevant experience will also be considered for the post.

Candidates should possess a good working knowledge of French and English and should also be conversant with the GLP regulations for Toxicology.

A realistic salary can be expected and relocation assistance will be available together with other financial benefits. All applications will be treated in the strictest confidence and should be sent directly to Mr J. Brault at the following address:

Synthelabo 58 Rue De La Glaciere 75013 Paris

W198(A)

## MEAT RESEARCH INSTITUTE SCIENTIFIC OFFICER

A vacancy exists in the Protein Section of the Meat Structure Division.

The project involves the study of enzymes capable of producing a tenderizing effect on meat.

The person who fills the post will be substantially responsible for studying the effect of neutral proteinases and should therefore be capable of working independently.

The work will involve protein fractionation techniques and enzymology, and experience in this field would be an advantage.

Qualifications: Degree in a relevant subject. Previous experience in protein chemistry and enzymology desirable.

Salary: £2,839 to £4,415. Starting salary depending on qualifications and experience. (Salaries are subject to review.)

Permanent, pensionable post. Application forms and further details from Personnel Officer, Meat Research Institute, Langford, Bristol BS18 7DY. Closing date for return of application forms July 12, 1979. 2348(A)

## POSTDOCTORAL POSITIONS IN VANCOUVER

Positions are currently available for studies on 1) the control of phosphatidylcholine biosynthesis in animals and 2) interactions between animal viruses and cell membranes. Stipends begin at \$12,200/annum. Apply with Curriculum Vitae, list of publications and names of 3 referees to Dr. Dennis E. Vance, Dept. Biochemistry, Univ. of British Columbia, Vancouver, BC V6T 1W5 Canada. W192(A)

## KENYATTA UNIVERSITY COLLEGE — KENYA

Applications are invited for the post of

### ASSOCIATE PROFESSOR

in the DEPARTMENT OF ZOOLOGY. Applicants must have a PhD in Zoology together with high level experience in University teaching and research. The appointee will be expected to teach undergraduates, supervise the work of post-graduate students and carry out personal research. Applications will be considered from persons specializing in any area of Zoology. The areas of specialization already represented in the Department are Ecology, Marine Biology, Freshwater Biology, Parasitology and Immunology, Arthropod Biology, Physiology, Ichthyology, Developmental Biology and Mammalogy. Salary scale: Associate Professor: K£3,864 to £4,488 pa (K£1 = £1.28 sterling). The British Government may supplement salaries by £5,784 pa (sterling) for married appointee and £3,876 pa (sterling) for single appointee (reviewed annually and normally free from tax) and provide childrens education allowances and holiday visit passages. Family passages; FSSU or SSSF; non-contributory medical scheme; subsidized housing/housing allowance; various allowances. Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to Registrar, Kenyatta University College, PO Box 43844, Nairobi, Kenya by 12 August 1979. Applicants resident in the UK should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 2319(A)

## AGRICULTURAL RESEARCH COUNCIL'S POULTRY RESEARCH CENTRE KING'S BUILDINGS WEST MAINS ROAD EDINBURGH EH9 3JS SCOTLAND

### NEUROPHYSIOLOGIST

Applications are invited for the post of HSO/SSO in the Ethology Department of the above Centre. The post is for a neurophysiologist to investigate the responses of alimentary receptors in relation to the control of food intake in poultry. The work will involve a study of receptors at the peripheral level using electrophysiological recording techniques. The work may be extended at a later date into the central nervous system. Applicants should have training in neurophysiology and an interest in sensory physiology and animal behaviour. (Ref. No. 799).

**Qualifications:** First or upper second class honours degree in an appropriate subject with at least two years relevant postgraduate experience and preferably with a PhD. Entry at SSO level requires at least 4 years postgraduate experience. **Salary:** In scale £4101 to £5,448 at HSO level and £5,154 to £6,898 at SSO entry. (Salaries currently under negotiation). Entry at the higher level requires at least 4 years post-graduate experience. Non-contributory superannuation scheme. 22 days annual leave plus 10½ public/privilege holidays. 1½% of salary is to be paid towards the Widows and Childrens Fund.

Application forms can be obtained from the Secretary at the above address. These forms should be completed and returned not later than 31st July 1979 quoting our reference number.

2289(A)

## KING'S COLLEGE HOSPITAL MEDICAL SCHOOL (University of London) Denmark Hill, London SE5 8RX JUNIOR MEDICAL LABORATORY SCIENTIFIC OFFICER 'B'

required for the Chest Unit in this Medical School. Work will involve contact with patients and assisting doctors with research into diseases of the lung. Minimum requirements, 5 'O' levels, 4 in Science subjects including maths, day release available. Post suitable for school leaver. Salary according to age, qualifications and experience.

Applications, in writing, giving full details should be sent to the Secretary of the Medical School at the above address. Closing date 13th July 1979.



# Nuclear Power Station Physicists

Challenging opportunities for young qualified physicists exist at Hinkley Point Power Station. The station situated on the Somerset coast near Bridgwater, in fine countryside, has four power reactors on the site which provide a wide range of technical, operational, engineering and safety problems. Applications are invited from recently qualified physicists, mathematicians or engineers to join a team dedicated to the solution of those problems.

We are looking primarily for young newly qualified staff for whom the salary would be in the range

**£4110 to £6035**

but suitable applicants with some relevant

experience would be considered for a more senior post within a salary range

**£5375 to £7050**

A self financing productivity payment is also payable and the salary scales are currently under review.

Terms and conditions are excellent and fringe benefits include relocation assistance where appropriate.

If you are interested please write for an application form AF/1 from the Personnel Manager, Bedminster Down, Bridgwater Road, Bristol BS13 8AN. Please complete and return quoting vacancy number R106/79/ N.

2359(A)

**Central Electricity  
Generating Board**  
South Western Region



## Leeds Area Health Authority (Teaching) Western District RESEARCH OFFICER (EPIDEMIOLOGY)

Research Officer (Scientific Officer Grade) is required to assist with studies of aetiology and distribution of lymphomas and leukaemias in the Yorkshire Region.

The study would be conducted from Leeds and involve the officer in interviewing and data analysis work in a variety of hospitals throughout Yorkshire. Applicants should have a broad education either in epidemiology, biology or social sciences and should possess a higher degree or have previous experience of population studies. The post which is currently available is for one year in the first instance renewable for a further two years.

Further particulars can be obtained from Dr. R.A. Cartwright, Yorkshire Regional Cancer Organisation, Cooridge Hospital, Leeds. Tel. Leeds 673411.

The salary will be on the Scientific Officer scale (N.H.S.) £3,486 to £4,275 per annum.

Applications obtainable from the Sector Personnel Officer, Leeds General Infirmary, Great George Street, Leeds LS1 3EX.

Closing date 16th July, 1979

2297(A)

## MRC CLINICAL RESEARCH CENTRE (NORTHWICK PARK HOSPITAL)

**WATFORD ROAD, HARROW, MIDDX. HA1 3UJ**

We require a PhD Pharmacologist and a Technician with a BSc or HNC both with experience in experimental cancer chemotherapy or in evaluating toxicity of antitumour drugs. The work, supported by a national institutes of health contract awarded to Dr. G. Gregoriadis is related to the use of Liposomes in cancer chemotherapy. The appointment will be for two years on a salary scale from £3,883 to £6,080 pa plus £502 LA.

Application form may be obtained from Mrs. J. Tucker-Bull, 01-864 5311 ext. 2685. Please quote ref. 111/1/2/NIH. Closing date July 14th, 1979.

2328(A)

## IMPERIAL COLLEGE UNIVERSITY OF LONDON DEPARTMENT OF CHEMICAL ENGINEERING AND CHEMICAL TECHNOLOGY

Applications are invited for the following position: Research Officer to work with the materials technology research group under the direction of Professor J. R. A. Pearson. The group is engaged in experimental and theoretical work on polymer chain dynamics (using relaxation, light scattering and computer simulation methods), polymer rheology, polymerisation reactors and polymer processing operations. Applicants should have a PhD in physical chemistry, physics or chemical engineering, previous experience with polymers being an advantage. Initial salary in range £4,250 to £7,145 pa plus £502 London allowance.

All applications, including a curriculum vitae and the names of two referees, should be sent to Dr. K. E. Bett, Department of Chemical Engineering, Imperial College, London SW7 2BY.

2272(A)

## CHARING CROSS HOSPITAL MEDICAL SCHOOL (University of London) LECTURER IN ANATOMY

Applications are invited for the above post. Candidates should possess a medical qualification or a higher degree in Anatomy or a cognate subject and special consideration will be given to those with an interest in neuroanatomy.

Salary on non-clinical Lecturer scale, i.e. £4,232 (£4,776 at age 26 or over) — £8,452 p.a. plus £502 London Weighting Allowance.

Applications in writing with names and addresses of two referees to The Secretary, Charing Cross Hospital Medical School, The Reynolds Building, St. Dunstan's Road, London W6 8RP, by 23rd July. (Ref: 016/8)

2353(A)

## MEDICAL RESEARCH COUNCIL VIROLOGY UNIT ENZYMOLOGIST PROTEIN BIOCHEMIST and NUCLEIC ACID BIOCHEMIST

Two vacancies exist for postdoctoral research scientists with experience in enzymology/protein biochemistry or nucleic acid biochemistry to work on a variety of molecular biological problems involving Herpes Simplex Virus and other mammalian virus systems.

Previous experience with virus systems is advantageous but not essential.

The appointment will be for three years. Salary on an appropriate point on Grade 2 of the non-clinical scientific staff scale. Applicants should send a Curriculum vitae giving full details of qualifications and experience and the names and addresses of three professional referees to: Professor J. H. Subak-Sharpe, Institute of Virology, University of Glasgow, Church Street, GLASGOW, G11 5JR, by 1st August, 1979.

2290(A)

## KENYATTA UNIVERSITY COLLEGE — KENYA

Applications are invited for the post of ASSOCIATE PROFESSOR IN THE DEPARTMENT OF CHEMISTRY. Candidates must have a PhD in Chemistry and considerable teaching experience at University level, at least at Senior Lecturer level. Further qualifications in Education or experience in the training of graduate teachers would be advantageous. The appointee will be expected to teach in the BEd and MSc programmes and to carry out and supervise research in his/her areas of specialisation. He/she will also be expected to be capable of providing academic and administrative leadership in the department. Salary scale: Associate Professor K£3,864 to £4,488 pa, (K£1 sterling = £1.28). The British Government may supplement salary by £5,784 pa (sterling) for married appointee or £3,876 pa (sterling) for single appointee (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages. Family passages; subsidised housing; SSSF or FSSU: non-contributory medical aid scheme. Detailed applications (2 copies) with curriculum vitae and naming 3 referees should be sent by airmail to the Registrar, Kenyatta University College, PO Box 43844, Nairobi, Kenya by 13 August 1979. Applicants resident in the UK should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address.

2298(A)

## CARDIOTHORACIC INSTITUTE LONDON

Applications are invited for two posts:-

- (1) Postdoctoral
- (2) Recent graduate

Appointments supported by a grant from the Multiple Sclerosis Society for a three year period to study mechanisms of cell accumulation and the action of drugs on this process, with particular reference to experimental allergic encephalitis. The work will involve *in vivo* experiments on cell accumulation, utilizing isotopic techniques recently developed in this laboratory. The graduate assistant can expect to be involved in extensive animal handling. The graduate appointee would be encouraged to register for a PhD. The postdoctoral appointee may be eligible for nomination to a lectureship in immunopharmacology.

For further information contact Dr J. Morley on 01-352 8121 Extn. 4194.

Please apply to Mr R.A. Perkins, Cardiothoracic Institute, Fulham Road, SW3 6HP with details of experience.

2365(A)

UNIVERSITY OF  
RHODESIA  
LECTURESHIPS/SENIOR  
LECTURESHIPS

Applications are invited for the following posts:

**BOTANY:** Applicants must have a relevant first degree and, preferably, a postgraduate qualification and relevant experience. Competence in one or more of the following fields will be advantageous: Anatomy and Morphology; Microbiology and Plant Physiology.

**CHEMISTRY:** The Department has lectureship vacancies in Physical and Inorganic Chemistry. Applicants must be suitably qualified with university or research experience. In addition the Department has responsibility for courses in Pharmaceutical Chemistry for B. Pharmacy degree students and, therefore, special interests or experience in pharmaceutical or medical applications of Chemistry would be an advantage.

**COMPUTING SCIENCE:** Applicants must have qualifications and experience in Computing Science. A knowledge of statistical methods will be an advantage.

**CROP SCIENCE — SCHOOL OF AGRICULTURE:** Applicants must have a relevant first degree and, preferably, an appropriate higher degree and relevant experience in Crop Science.

**ELECTRICAL AND MECHANICAL ENGINEERING:** Applications are invited from persons with Engineering Degrees and postgraduate experience for Lectureship posts in: (a) the Department of Electronic and Power Engineering (experience desirable in Electronic Power Systems, Electrical Machines and Electromagnetics); (b) the Department of Mechanical Engineering (Experience desirable in Thermodynamics, Heat Transfer and Mechanical Design).

Subject to University permission earnings up to a specified limit per annum for consultancy may be retained in full in addition to basic salary.

**ZOOLOGY:** Applicants must have a relevant first degree. A higher degree and postgraduate experience is desirable. Preference may be given to applicants with competence in one or more of the following fields: Vertebrate Zoology; Terrestrial Ecology; Genetics and Parasitology.

**SALARY SCALES AND CONDITIONS OF SERVICE.**

Salary (Approx. Stg. Equiv.):  
Senior Lecturer £7,088 × 275 to £7,913 × 292 to £9,373 p.a.  
Lecturer Grade I £6,429 × 242 to £7,397 p.a.  
Lecturer Grade II £3,969 × 208 to £5,009 × 242 to £6,219 p.a.

Commencing salary according to qualifications and experience. Family passages and allowances for transport of effects. Installation Grant and Installation Loan; Sabbatical Leave and Contact Visits; Superannuation and Medical Aid Schemes. For persons recruited from outside Zimbabwe-Rhodesia, unfurnished accommodation on or near the Campus and close to good junior and senior schools, is guaranteed for up to three years.

Appointments may be on permanent pensionable terms or for a fixed term of 1 or 2 years in the first instance. In the case of 1 or 2 year contracts a non-pensionable salary is payable on the above scales plus a 10% supplement.

At present, immigrants and persons on temporary contract, are exempted from military service for two years from the date of their arrival in Zimbabwe-Rhodesia.

Further particulars and details of application procedure may be obtained from the Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF.

Closing date for the receipt of applications is 18 July 1979.

British subjects considering applying for posts in Rhodesia are urged to consult the Foreign and Commonwealth Office (telephone 01-233 8727) or their nearest British Consular Office. 2318(A)

# OVERSEAS DEVELOPMENT

KNOW-HOW-vital to developing countries

## Rice Agronomist

Tanzania

Required to work in Southern Tanzania with responsibility for executing a rice research programme. Will be stationed at Naliendele Agricultural Research Institute and will provide the main source of professional advice on rice production with priority being given to the areas selected for inclusion in the Tanzania/British Joint Programme. Will be assisted by a Field Officer and two Field Assistants provided by the Ministry of Agriculture and will directly supervise their work. Will also advise on a suitable staff training programme, including in service and overseas training. At least five years experience of irrigated rice production and experimentation required.

Appointment 2 tours of 1 year. Salary (UK taxable) in senior range above £10,000 pa plus tax-free overseas allowances in range £1,400 to £3,570 pa and £380 to £760 pa (Ref 331X)

*The post is wholly financed by the British Government under Britain's programme of aid to the developing countries. In addition to basic salary and overseas allowances other benefits normally include paid leave, free family passages, childrens education allowances and holiday visits, free accommodation and medical attention. Applicants should be citizens of the United Kingdom.*

*For full details and application form please apply, quoting reference stating post concerned, and giving details of age, qualifications and experience to:—*



Appointments Officer,  
OVERSEAS DEVELOPMENT ADMINISTRATION,  
Room 301, Eland House,  
Stag Place, London SW1E 5DH.

HELPING NATIONS HELP THEMSELVES

2341(A)

## UNIVERSITY OF KEELE

### DEPARTMENT OF GEOLOGY

#### Departmental Demonstratorships

Applications invited for two full-time posts of Demonstrator from 1st October 1979, for one year in first instance, to assist with teaching of all aspects of Geology at an elementary level together with advanced courses in fields specified below. Postgraduate and/or industrial experience and a current British driving licence would be advantageous.

Post (a) Demonstrator in mineralogy, igneous and metamorphic petrology and map work.

Post (b) Demonstrator whose major interest is in clastic or carbonate sedimentology.

Initial salary £3,775 on Grade 1B scale, with membership of the Universities Superannuation Scheme.

Further details and application forms from The Registrar, The University, Keele, Staffs., ST5 5BG, to whom application forms should be returned not later than Friday 6th July 1979. (Previous applicants are not required to re-apply.) 2278(A)

## UNIVERSITY OF DURHAM

### DEPARTMENT OF BOTANY

Applications are invited from candidates with biochemical experience for the post of Senior Experimental Officer tenable from 1 October 1979. Duties include both demonstrating and research plus responsibility for the operation of some physiological and biochemical equipment.

Salary on Range 1A £4,333 to £7,521 pa plus superannuation.

Applications (3 copies), naming three referees should be sent by 4 July to the Registrar and Secretary, Science Laboratories, South Road, Durham, DH1 3LE, from whom further particulars may be obtained. 2275(A)

## CSIRO AUSTRALIA

### Postdoctoral Research Fellow

#### Division of Mineral Chemistry

#### Port Melbourne Victoria

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees — 2,400 of whom are research and professional scientists — located in Divisions and Sections throughout Australia.

**GENERAL:** The Division conducts research in the fields of solid-state and surface chemistry, metallurgy and electrochemistry. The research program on high energy density batteries for traction applications is being expanded under sponsorship from the National Energy Research, Development and Demonstration Council.

**DUTIES:** To participate in a research program to develop nickel-zinc secondary batteries.

**QUALIFICATIONS:** A PhD or equivalent in a relevant area of chemistry or chemical engineering. Experience in electrochemistry or battery technology would be an advantage.

**SALARY:** Research Scientist A\$15,422 to A\$18,904 p.a.

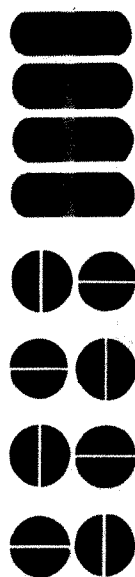
**TENURE:** The appointment will be for 2½ years with a possibility of extension.

Applications IN DUPLICATE, stating full personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 601/307 should reach:—

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, 10-16 Maltravers Street, London WC2R 3EH, by 27th July, 1979.

Applications in USA and Canada should be sent to:— The Counsellor Scientific, Embassy of Australia, 1601 Massachusetts Avenue, N.W., Washington, D.C., 20036, U.S.A.

2352(A)



# Cardiovascular Pharmacologists

Applications are invited from experienced pharmacologists for the following positions which have arisen within the highly successful cardiovascular sections of our Pharmacology Department.

## 1. A Graduate or PH.D. Pharmacologist

is required to lead a small team of technicians developing continuous recording techniques in conscious animals, in order to study the haemodynamic effects of new drugs. The successful candidate will be a skilled practical worker and will have at least 3 years' experience of cardiovascular techniques, preferably in conscious animals. There will be scope for basic research in associated areas.

## 2. A Graduate or PH.D. Pharmacologist

is required to join an established team of graduates and technicians, whose immediate objective is the identification of novel mechanisms to further the development of new antihypertensive agents. The ideal applicant will have at least 2 years' postgraduate experience and a thorough knowledge of the cardiovascular and autonomic nervous systems. He or she must be able to work independently within a specific area of investigation.

These are challenging and rewarding positions, and the people appointed will be expected to make a substantial contribution towards the creative effort of the department.

We offer a competitive salary commensurate with experience, plus a Ware Allowance. The successful candidates will participate in a non-contributory pension and life assurance scheme, as well as bonus and productivity schemes. Other benefits include a subsidised canteen, a flourishing sports and social club and (where appropriate) assistance with relocation to this most attractive area of Hertfordshire.

Please write, or telephone, for an application form to: Dr. D.J. Humphreys, Personnel Officer, Glaxo Group Research (Ware) Ltd., Ware, Hertfordshire SG12 0DJ. Tel: Ware 3232 2362(A)

# Glaxo Group Research Ltd.

## PAISLEY COLLEGE

Due to the continuing expansion of its CNAA degree programme, Paisley College, a Scottish Central Institution, invites applications for the following post:—

## LECTURER IN BIO-CHEMISTRY Department of Biology

Applicants should have a good honours degree in Biochemistry and post-graduate research experience.

The successful candidate will have an interest in Pharmacology and will be involved in the teaching of Biochemistry at HNC, HND, BSc (Biology/Dietetics) and honours levels. In addition a contribution to the teaching of Pharmacology to HNC and BSc (Dietetics) is expected. Research is encouraged.

## SALARY SCALE Lecturer 'A' £4,422/£8,391.

Application forms and further particulars may be obtained from The Personnel Officer, Paisley College of Technology, High Street, Paisley (Tel No. 041 887 1241), to whom completed application forms should be returned within ten days of the appearance of this advertisement. 2306(A)

## UNIVERSITY OF CAMBRIDGE

### DEPARTMENT OF PHARMACOLOGY

Applications are invited for the office of  
UNIVERSITY LECTURER IN  
THE DEPARTMENT OF  
PHARMACOLOGY.

The initial appointment will be for three years from 1 October 1979 with the possibility of reappointment to the retiring age. The pensionable stipend for a University Lecturer will be on a scale of £5,850 rising by twelve annual increments to £9,000 a year with initial placing above the minimum where appropriate. There is no grade of Senior Lecturer. A grant is made towards removal expenses.

Candidates should send twelve copies of their application together with the names of not more than three referees to Mr G. R. Anderson, General Board Office, The Old Schools, Cambridge, CB2 1TT, from whom further information can be obtained, to arrive not later than 15 August 1979. 2292(A)

LABORATORY SCIENTIFIC OFFICER required for two years to join a research team investigating mammalian meiosis. HNC or equivalent qualification required. Applications should be sent to the Administrative Assistant, Paediatric Research Unit, The Prince Philip Research Laboratories, Guy's Tower, Guy's Hospital, London Bridge, SE1 9RT, as soon as possible. 2284(A)

## POSITION OPEN BIOMEDICAL MASS SPECTROSCOPIST (PhD)

wanted immediately. Duties include planning and execution of multiple research programs involving application of gas chromatographic mass spectrometric techniques to biomedical and environmental problems. Salary \$15,000/annum. Please send resumes to: The Oregon Graduate Center, 19600 NW Walker Road, Beaverton, OR 97005 Attn: Joanne Brink, Personnel Administrator AA/EEO. W193(A)

## MEDICAL RESEARCH COUNCIL RADIOBIOLOGY UNIT CYTOGENETICS

A vacancy exists for a scientist to work in a group investigating the effects of ionizing radiation upon chromosomes in a variety of cells and tissues.

Applicants should have a good honours degree, and at least 3 years post-graduate research experience. They should be conversant with modern cytogenetic techniques and some knowledge of tissue culture is desirable.

Usual Medical Research Council conditions of service. Salary according to age and qualifications. Applications in writing to: The Administrator, M.R.C., Radiobiology Unit, Harwell, Didcot, OXON OX11 0RD, quoting reference JRKS/1. 2 2 8 0 (A)

## UNIVERSITY COLLEGE CARDIFF

Applications are invited for the post of  
POST DOCTORAL  
RESEARCH ASSISTANT

in the DEPARTMENT OF CHEMISTRY (Inorganic). Research would be in co-ordination chemistry, probably in one of: Raman spectroscopy, crystallography of stereo-chemistry, reactions of ligands and mechanisms, biological aspects. Some teaching duties will be required. Salary range: £4,232 to £5,321 p.a. Duties to commence as soon as possible.

Applications (2 copies), together with the names and addresses of two referees should be forwarded to the Vice-Principal (Administration) and Registrar, University College PO Box 78, Cardiff CF1 1XL. Further details available from Professor Gillard, Department of Chemistry (Inorganic), Cardiff 44211 Ext. 2173. Closing date 31st July 1979. Reference 1835. 2271(A)

## NATIONAL VEGETABLE RESEARCH STATION ASSISTANT NEMATOLOGIST

A SCIENTIFIC OFFICER is required to assist with work on the nematode pests of vegetables.

The appointment will be in the grade of Scientific Officer salary scale £2,839 per annum rising by annual increments to £4,415. (Salary review 1 April 1979 pending).

Minimum qualifications required is a degree or equivalent in Biological Science and experience in Nematology would be an advantage.

Non-contributory employers' additional pension scheme.

Full particulars and application form (to be returned by 18 July 1979) from the Secretary, National Vegetable Research Station, Wellesbourne, Warwick CV35 9EF. 2316(A)



**PORTSMOUTH  
POLYTECHNIC  
BIOCHEMISTRY/  
MOLECULAR BIOLOGY**

A Lecturer II/Senior Lecturer is required to undertake teaching and research duties in this field.

The person appointed will take overall responsibility for the teaching of biochemistry and molecular biology within the multi-disciplinary Biomolecular Science BSc. course (3 years full-time, Honours) and will personally teach a major proportion of the material.

Facilities for research are extensive. The existing research group emphasises a multi-disciplinary approach to the structure and function of chromatin and is supported by SRC and CRC.

Salary scale: £4,101 to £6,051 (efficiency bar) to £7,572 per annum (under review).

Further details and application forms from the Staff Officer, Portsmouth Polytechnic, Alexandra House, Museum Road, Portsmouth, PO1 2QQ, to whom completed applications should be returned by 20th July 1979, quoting ref. no. S56.

2268(A)

**CHELSEA HOSPITAL FOR  
WOMEN**

Dovehouse Street, London SW3

The Semiology Laboratory at the Chelsea Hospital for Women require a

**MEDICAL LABORATORY  
SCIENTIFIC OFFICER**

with experience in bacteriology or immunology.

The work involves investigation of subfertile couples, seminal analysis, freezing sperm and the detection of sperm antibodies in male and female by macro and micro techniques. Experience with tissue typing would be useful.

Further information and application form obtainable from The Administrative Assistant, Queen Charlotte's Maternity Hospital, Goldhawk Road, London W6 0XG. Tel: 01-748 4666 ext 204.

2327(A)

**UNIVERSITY OF BRISTOL**

Department of Biochemistry

**POSTDOCTORAL  
RESEARCH ASSISTANT**

Applications are invited for a postdoctoral research assistant to join a group investigating the hormonal regulation of glutamine metabolism in liver. The appointment is for a maximum of three years and is funded by the Medical Research Council. Salary is within the University Range 1A — £4,232 to £7,145 per annum (scale subject to revision from 1st October, 1979). Some experience in protein separations techniques would be advantageous.

Applications, enclosing a curriculum vitae and the names and addresses of two referees should be sent to Dr. J. D. McGivan, Department of Biochemistry, Medical School, University Walk, Bristol BS8 1TD before July 15th, 1979.

2296(A)

**ROYAL FREE HOSPITAL  
SCHOOL OF MEDICINE  
University of London**

Department of Biochemistry and Chemistry

**POSTDOCTORAL  
RESEARCH ASSISTANT**

Bio-physical chemist required to join research group working on membrane structure and function. Experience of surface techniques or material science would be a particular advantage. The post is tenable for up to 3 years, supported by the Science Research Council. Initial salary within range £4,261 to £4,805 plus £502 London allowance.

Applications, naming two referees, to the School Secretary, R.F.H.S.M., 8 Hunter Street, London, WC1N 1BP as soon as possible.

2276(A)



**european space agency**

The European Space Agency has the following vacancies in the Space Science Department at its Technological Centre in Noordwijk, the Netherlands:

**Astronomers**

Applications are invited from U.V. and optical astronomers. Applicants should either have a strong background in advanced detector systems, with experience in their application to astronomy, or they should be observers with an extensive astrophysical background and experience in using advanced space and ground based instruments. Duties will include scientific project work associated with possible future and on-going space astronomy projects together with joint research using IUE and an advanced photon counting system.

**Space Plasma Physicists**

Applications are invited from physicists with experience in particle, field and wave measurements in a magnetized plasma — in space or in the laboratory. Interested candidates should have a background in experimental techniques in this field and preferably also have experience with data analysis. Duties will partly be scientific project work related to studies and on-going projects where elements of space plasma physics are included, and partly research related to experiment development and analysis of data from on-going projects such as GEOS and ISEE.

Applications for these posts should be sent, as soon as possible, to the Head of Personnel, ESTEC, Zwarteweg 62, Postbus 299, AG 2000, Noordwijk, the Netherlands.

W189(A)

**UNIVERSITY OF EXETER  
LECTURER IN  
COMPUTER SCIENCE**

Applications are invited for a lectureship in the Department of Computer Science, tenable from 1 October 1979.

Candidates should be qualified persons working in any area of computer science. Specializations which are of particular interest, but which are not intended to make up an exhaustive list, are: data-base computation and theory; artificial intelligence; computer architecture and systems; theory of programming languages and semantics; operating systems; distributed computing and networks.

Commencing salary will be within the range £4,232 to £6,368 pa (under review) on the scale £4,232 to £8452 pa (under review). The post is subject to a probationary period not exceeding three years with the prospects of permanency thereafter.

Further particulars may be obtained from Miss Doreen Watson, University of Exeter, Northcote House, Exeter, EX4 4QJ, to whom applications (8 copies) should be forwarded by 23 July 1979. Please quote reference No 3216.

People who have applied for a similar post earlier this year will be automatically considered for this present post.

Further information may be obtained from Professor J. A. Campbell, Department of Computer Science (Ext. 216).

2313(A)

**UNIVERSITY COLLEGE GALWAY, IRELAND**

**DEPARTMENT OF PHYSICS**

Arising out of the introduction of an optional honours physics degree in applied physics and electronics, an initial appointment in one of the following specialities is to be made.

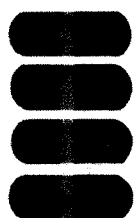
- 1) Physicist or Electronic Engineer with broad experience in hardware and software computing or
- 2) Electronic/Electrical Engineer or Physicist with experience in control systems and/or micro-electronics. Industrial experience would be an advantage.

The salary will be in the range £5,000 to £8,000 p.a.

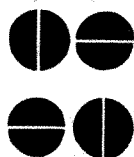
The appointment will be for twelve months and will not be renewable. The college however, hopes to advertise several appointments in this area in 1980 at either Junior Lecturer (£4,852 to £6,706, under review) or Lecturer (£7,220 to £9,530, under review).

For further information please contact the Registrar (Telephone Galway 7611, ext. 192, telex 8823). Closing date for receipt of applications is July 6th 1979.

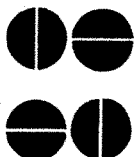
2339(A)



## Director of Biology Ware, Herts.



Applications are invited from experienced biochemists or pharmacologists to be responsible to the Managing Director for the scientific standards of work carried out in the Pharmacology and Biochemistry Departments. This includes testing and evaluating research compounds as well as basic research.



In addition, the position involves directing multi-disciplinary research projects, and there is considerable interaction with senior people in related disciplines. Consequently, sufficient knowledge of other areas is necessary to enable the successful applicant to make a full contribution to the research and development activity.

Candidates must have extensive industrial experience of pharmaceutical R&D in a pertinent biological discipline. It is unlikely that people below the age of 35 will have the required knowledge or experience for the position.

The salary for this senior position is negotiable and a company car will be provided. Our modern, well-equipped laboratories are set in pleasant surroundings in Hertfordshire, and relocation assistance will be available if appropriate. Other benefits include non-contributory pension scheme and the opportunity to participate in Group profitability.

Please forward your curriculum vitae, in confidence, to: Ian Collins, Ph.D., Personnel Manager, Glaxo Group Research Ltd., Ware, Herts. SG12 0DJ. 2329(A)

# Glaxo Group Research Ltd.

## UNIVERSITY OF MANCHESTER DEPARTMENT OF MEDICAL BIOCHEMISTRY POST-DOCTORAL RESEARCH ASSOCIATE

Applications invited for this post (available up to three years from October) to work with Dr C. A. Shuttleworth and Dr M. E. Grant on "The Role of Non-Collagenous proteins in the Periodontal Ligaments". Some experience in the fields of connective tissue biochemistry, protein chemistry, tissue culture or embryogenesis would be an advantage. Starting salary in the range £4,333 to £4,910 p.a. Superannuation. Applications (three copies) including curriculum vitae and the names of two referees should be sent to Dr C. A. Shuttleworth, Department of Medical Biochemistry, University of Manchester Medical School, Manchester M13 9PT as soon as possible. 2312(A)

## UNIVERSITY OF CAMBRIDGE ZOOLOGY DEPARTMENT

### Research Assistant

To collaborate with Dr. M. Burrows on the morphology or physiology of local interneurons in insects. Experience of light or electron microscopical techniques, or of neuro-physiological techniques an advantage. The appointment, to begin as soon as possible, is short term, approx. 18 months. Salary according to age and experience to £4776 max. (under review). Applications (c.v. and names of two referees) to Dr. M. Burrows, Zoology Department, Downing Street, Cambridge CB2 3EJ by 31st July, 1979. 2364(A)

## MANCHESTER AREA HEALTH AUTHORITY (T) CENTRAL DISTRICT SENIOR CYTOGENETICIST

The expanding Regional Laboratory which deals with blood, fibroblast and amniotic material is located within the University Department of Medical Genetics. This Department also provides Genetic Counselling and HLA typing services and there is excellent clinical liaison.

Only candidates holding or eligible for an NHS Senior Cytogeneticist post and having extensive experience of Medical Cytogenetics should apply.

Applications with full details of experience and qualifications and the names of two referees should be sent to Dr Rodney Harris, Department of Medical Genetics, St. Mary's Hospital, Manchester M13 0JH. 2308(A)

## UNIVERSITY COLLEGE LONDON RESEARCH ASSISTANT

Research Assistant required to assist physical anthropology staff in research and teaching duties. The post will also involve curation of the physical anthropology teaching material collection, routine computer/clerical tasks and assistance in specific research projects. A first degree in a biological science and an interest in physical anthropology/human biology is essential. The successful candidate may be given an opportunity to conduct research leading to a higher degree. Tenable from October 1979. Salary range £3,775 to £4,910 plus £502 London allowance. Applications to Assistant Secretary (Personnel) University College London, Gower St, London WC1E 6BT. 2320(A)

## UNIVERSITY OF LIVERPOOL BIOENGINEERING AND MEDICAL PHYSICS UNIT RESEARCH ASSISTANT

Applications are invited for the post of Research Assistant/Senior Research Assistant to work on a research project concerned with the development of an artificial blood vessel.

The project has been running for three years and has led to a prototype vessel which is at present undergoing thorough evaluation, leading eventually to commercial exploitation.

The research assistant will be involved in the application and development of small scale production and testing apparatus, as well as being innovative in making improvements necessary for future generations of prosthesis. He/she will be expected to utilize his/her own specialities and interests in the research and development of the improved product.

The post is most suitable for someone with a degree in a related physical science, an interest in bioengineering and possibly research experience. Any queries regarding the post should be made to Mr R. Clarke, 051-709-6022, ext. 2292.

The initial salary will be on the scale £3,689-£5,321 p.a., or £4,232-£4,776 p.a., for candidates with a Ph.D.

Applications, together with the names of three referees, should be received not later than 20th July, 1979 by the Registrar, The University, P.O. Box 147, Liverpool, L69 3BX. Quote Ref: RV/673. 2357(A)

## UNIVERSITY OF SALFORD DEPARTMENT OF CHEMISTRY AND APPLIED CHEMISTRY LECTURER IN ORGANIC CHEMISTRY

Applications are invited for the above post from well qualified candidates with teaching and research interests in the field of pure and applied chemistry. The post is available from 1 October, 1979.

Salary scale £4,232 to £8,452 p.a. USS benefits.

Application forms and further particulars are available from the Registrar, University of Salford, Salford M5 4WT (tel: 061-736 5843 ext. 215) to whom completed applications should be returned by 27 July, 1979, quoting reference no: CH/260/N. Informal enquiries may be made to Professor H. Suschitzky. 2326(A)

## UNIVERSITY OF LIVERPOOL

### Department of Oceanography Senior Research Assistant/ Research Associate

Applications are invited for the post of Senior Research Assistant/Research Associate to investigate the fine structure and microstructure of the upper oceanic layers. Candidates should possess a PhD with preferably some experience in numerical modelling. The project is sponsored for three years by the Ministry of Defence at an initial salary within the range £4,776 to £6,368 per annum, depending on age and experience.

Applications, together with the names of three referees should be received not later than 1st August 1979, by the Registrar, The University, PO Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/671. 2355(A)

## ODENSE UNIVERSITY DENMARK

A new position as Professor in Photosynthesis has been created in the Institute of Biochemistry, and is available for occupation as soon as possible.

It is desired to appoint a person whose research activities are experimental investigations of the functions of the photosynthetic membrane systems in eukaryotes and/or prokaryotes. A part of the current research in the Institute is concerned with electron transport in respiratory and photosynthetic systems, and the Institute is well equipped with special apparatus for this type of work.

The successful applicant will be expected to participate in the teaching programme of the Institute, which includes elementary courses common to science and medical students, advanced courses for advanced students and supervision of advanced project work.

Further information is obtainable from Institute of Biochemistry, Odense University, telephone (09) 15 86 00, ext. 2441.

The application should contain information about the candidate's teaching experience. It is expected that the successful applicant will eventually be able to teach in Danish.

The employment field covers the Ministry of Education and the institutions under it. The wage frame is 37, and the salary amounts to Danish Kroner 230.520,24 a year inclusive of bonuses as per the October 1, 1978.

A professional selection committee will discuss the applications and their recommendation will be sent out to all applicants in its complete form.

Application in 5 copies, marked "Position No. 745", enclosing curriculum vitae and documentation for professional and pedagogical activities must be made to the Queen and sent together with all enclosures also in 5 copies to: Journalkontoret, Odense University, Campusvej 55, DK 5230 Odense M, Denmark, so that we will receive it by October 1, 1979 at the latest. W173(A)

# AGRICULTURAL RESEARCH COUNCIL

## UNIT OF NITROGEN FIXATION

A vacancy has arisen for a Scientific Officer to work in our Chemistry Department.

The work will involve the development of catalytic systems for the reduction of molecular nitrogen. The successful applicant will work with a minimum of supervision and after consultation will be required to design new experiments. Applicants should have a Degree in Chemistry.

Salary in a scale £2,839 to £4,415 (under review from 1 April 1979) depending upon qualifications and experience. Non contributory superannuation scheme.

Applications in writing to the Secretary at the ARC Unit of Nitrogen Fixation, University of Sussex, Brighton BN1 9RQ with curriculum vitae and the names of three referees. Closing date 12/7/79.

2324(A)

# REPRODUCTIVE BIOLOGIST

## MCGILL UNIVERSITY Montreal

### DIVISION OF REPRODUCTIVE BIOLOGY DEPARTMENT OF OBSTETRICS AND GYNECOLOGY

invites applications for an Assistant Professor position. Candidates must hold Ph.D. or M.D. degrees, have at least two years of post-doctoral training and have documented research interests in some aspect of reproductive biology (e.g., neuro-endocrinology, gonadal function, fetal growth, hormone receptors, etc). Candidates will be expected to establish their own research group and to compete for outside grant support. Personal salary and starting date negotiable; teaching commitments commensurate with experience. Closing date for applications: September 1, 1979. Send curriculum vitae, list of publications and three letters of reference to: Dr K. B. Ruf, Research Laboratories, Women's Pavilion, Royal Victoria Hospital, 687 Pine Avenue West, Montreal H3A 1A1, Canada.

W196(A)

# THE UNIVERSITY OF TORONTO ASSISTANT PROFESSORS IN BIOCHEMISTRY

Applications are invited for two positions of Assistant Professor in the Department of Biochemistry (one to be located in the Playfair Neurosciences Unit) to take effect from July 1, 1980. Both appointees will be on a two-year contract with possibility of renewal for a further three years. The successful applicants will be required to take part in the teaching programs of the Department and to develop a research program. The appointment to the Playfair Unit will be made in the area of membrane or receptor biochemistry; the other position has no field restrictions. The Department is involved in teaching Biochemistry to undergraduates in the Faculties of Medicine, Dentistry, Arts and Science, and Nursing, as well as graduate students. The minimum salary for both positions will be \$18,000.00.

Applicants with suitable qualifications should send a curriculum vitae and the names and addresses of three referees to: Chairman of the Search Committee, Department of Biochemistry, University of Toronto, Toronto, Canada, M5S 1A8, to reach him not later than September 30, 1979.

W182(A)

# UNIVERSITY OF LEEDS Grade 5 Technician

An experienced Laboratory Technician is required immediately for the Department of Obstetrics and Gynaecology (Leeds Maternity Hospital). The technical work is concerned mainly with immunological aspects of human reproduction and requires knowledge of immunologic methodologies. The holder of this post is also responsible for the routine supervision of the laboratory, including ordering and checking supplies and equipment. Applicants should possess an HNC or equivalent qualifications and have had about seven years relevant experience. Salary on the scale £3,474 to £4,056 p.a. Applications should be made in writing to Professor J. S. Scott, Department of Obstetrics and Gynaecology (Leeds Maternity Hospital) University of Leeds, 17 Springfield Mount, Leeds LS2 9NG.

2333(A)

# EAST BIRMINGHAM HOSPITAL REGIONAL CYTOGENETICS LABORATORY

Applications are invited for the post of Scientific Officer. Experience in medical cytogenetics including cell tissue culture desirable but not essential. Salary range depending upon experience and qualifications £2,991 to £4,899 per annum.

Application forms and job description obtainable from Regional Cytogenetics Laboratory, East Birmingham Hospital, Bordesley Green East, Birmingham B9 5ST. Tel. 021 772 4311 ext. 4354.

2335(A)

# DEPARTMENT OF MORPHOLOGY UNIVERSITY OF GENEVA MEDICAL SCHOOL Geneva, Switzerland

invites applications for a position of

## RESEARCH ASSOCIATE

in Developmental Biology

Candidates should be M.D. or Ph.D. with experience of teaching embryology to medical students and be able to set up an active research unit in the Department. Ability to teach in French is mandatory. Salary, before income tax, starts at Sfr. 57,000 per annum. Send complete résumé to: Dr L. Orci, Chairman, Department of Morphology, Medical School, rue Ecole de Médecine, 1211 Geneva 4, Switzerland.

W197(A)

# FORINTEK CANADA CORPORATION WESTERN FOREST PRODUCTS LABORATORY

Wood Biodeterioration and Protection — a PROFESSIONAL MYCOLOGIST is required immediately to join a highly qualified research team in Vancouver, Canada. Applicants should have a Ph.D. plus several years experience with wood-inhabiting fungi. A knowledge of decay problems in buildings would be desirable. A demonstrated ability to do applied research and to communicate both to industry and the general public will be required. Salary negotiable from 25,000 dollars, depending on qualifications and experience. Applications, with two references, to Dr R. S. Smith, Forintek Canada Corp., Western Forest Products Laboratory, 6620 N.W. Marine Drive, Vancouver, B.C., Canada V6T 1X2.

W199(A)



# NURSING PRACTICE RESEARCH UNIT

## NORTHWICK PARK HOSPITAL & CLINICAL RESEARCH CENTRE

# Project Leader

Salary in the range £3,883 to £6,555 p.a. plus £502 UGC London Weighting Allowance

(Starting salary will depend upon qualifications and experience.)

Applications are invited for the new post of Project Leader in the developing national Nursing Practice Research Unit which has been established by the Department of Health and Social Security at Northwick Park Hospital. Applicants should be science graduates, with suitable research experience, and preferably hold a nursing qualification. The appointment is initially for a three-year period.

The Project Leader will primarily be responsible for developing a programme of research into the treatment of Pressure Sores. In addition, he/she will contribute to the broad development of the Unit as a national centre for research into clinical nursing practice. This will include devising methods and techniques for clinical nursing research and assisting with the training of nurses and others who are interested in nursing practice research. The Unit will also act as a centre for the collation and dissemination of information about research into nursing practice.

Interested applicants are welcome to contact Dr. Rosemary Crow, Director, Nursing Practice Research Unit, for informal discussion before submission of application. Telephone 01 864 5311 ext. 2881.

Further particulars and application forms can be obtained from the Area Personnel Department, Brent and Harrow Area Health Authority, Signal House, Lyon Road, Harrow, Middlesex. Telephone 01 863 9111 ext. 41.

Closing date for applications is 20th July, 1979.

2349(A)

# THE MAX-PLANCK-INSTITUT FÜR ZÜCHTUNGSFORSCHUNG

invites applications for the post of

## MOLECULAR GENETICIST

to take part in a research programme aimed at elucidating the genetics of the interactions between plant and bacterium in symbiotic nitrogen fixation. The project will involve investigation of legume and *Rhizobium* genomes in controlling specificity in the symbiosis. The successful applicant will have had post-doctoral experience with plasmid isolation and characterization.

Two types of appointment are possible: for a fixed period of up to five years or for an indefinite period. Both carry remuneration on the German BAT scales 2a to 1a. Applicants should send a curriculum vitae and the names and addresses of three referees to

J. Schell, Director  
Max-Planck-Institut für Züchtungsforschung  
5000 Köln 30 (Vogelsang)  
Federal Republic of Germany

before October 1, 1979.

W191(A)



Brent & Harrow Area Health Authority Brent Health District  
Central Middlesex Hospital

## Medical Laboratory Scientific Officer

CLINICAL INVESTIGATION DEPARTMENT

We require either a qualified M.L.S.O. or a B.Sc. Graduate in Medical Sciences to fill the above post shortly to become vacant. Duties cover basic laboratory work specialising in Radio Immuno Assay techniques. Previous experience in this field or within a Biochemistry orientated discipline would be preferable.

**Hours:** 38 per week  
**Salary:** Qualified M.L.S.O. £3,615 to £5,034 inclusive.

**Please contact** Mrs. Janice Andrews, Personnel Department, Central Middlesex Hospital, Acton Lane, London NW10 7NS Telephone: 01-965 5733 ext. 656. 2342(A)

REQUIRED BY THE  
FOOD AND AGRICULTURE  
ORGANIZATION OF THE  
UNITED NATIONS, ROME, ITALY

## CROP PROTECTION SPECIALISTS

(Phytopathologists, Entomologists, Weed Scientists)

to be stationed in Sahelian countries, serving in a large-scale project on Integrated Pest Management for Basic Food Crops implemented with the assistance of FAO by the Inter-States Permanent Committee for Drought Control in the Sahel (CILSS).

**ESSENTIALS:** Ph.D. in Plant Protection Sciences. At least 7 years of post-graduate experience in pest management. Good working knowledge of English or French.

**SALARY:** dependent on qualifications/seniority, net tax-free including the usual International Civil Service allowances. Please send detailed curriculum vitae quoting "TF/RAF/128(USA)-comm." to Mr T. Eshetu, Manpower Planning, AGO, FAO, Via delle Terme di Caracalla, 00100 Rome, Italy. W184(A)

### UNIVERSITY OF EXETER LECTURER IN COMPUTER SCIENCE

Applications are invited for a lectureship in the Department of Computer Science, tenable from 1 October 1979.

Candidates should be qualified persons working in any area of computer science. Specializations which are of particular interest, but which are not intended to make up an exhaustive list, are: data-base computation and theory; artificial intelligence; computer architecture and systems; theory of programming languages and semantics; operating systems; distributed computing and networks.

Commencing salary will be within the range £4,232 to £5,048 pa (under review) on the scale £4,232 to £8452 pa (under review). The post is subject to a probationary period not exceeding three years with the prospects of permanency thereafter.

Further particulars may be obtained from Miss Doreen Watson, University of Exeter, Northcote House, Exeter, EX4 4QJ, to whom applications (8 copies) should be forwarded by 23 July 1979. Please quote reference No 3216.

People who have applied for a similar post earlier this year will be automatically considered for this present post.

Further information may be obtained from Professor J. A. Campbell, Department of Computer Science (Ext. 216). 2313(A)

### UNIVERSITY COLLEGE LONDON

#### DEPARTMENT OF CHEMISTRY

Applications are invited for an SRC Post-Doctoral Assistant available for one year from October 1st 1979. The successful applicant will investigate the ionisation potentials of organic molecules and the appearance potentials of fragment ions with the object of accurately determining the heats of formation of such ions. The apparatus consists of a double hemisphere electron energy selector, a quadrupole mass filter and ion counting equipment. The instrument is controlled by a PDP8 computer. Salary in range £4,261 to £4,805 plus £502 London allowance; USS. Applications consisting of curriculum vitae and names and addresses of two referees should be sent as soon as possible to Professor Maccoll, Dept. of Chemistry, University College London, 20 Gordon St, London WC1H 0AJ. 2273(A)

### UNIVERSITY OF READING POSTDOCTORAL RESEARCH ASSISTANT

GEOCHEMIST required as soon as possible, for a fixed term to be determined, for research on volatiles in magmas and minerals. The project funded for three years by the NERC is supervised by Professor D. K. Bailey and is to apply new techniques to the extraction and analysis of gases from glassy lavas, and volatile-bearing minerals, with the aim of elucidating igneous and ore-forming processes. Applicants should already have a doctorate (or be submitting a thesis) in geochemistry. Experience in experimental petrology, mass spectrometry or gas chromatography will be an advantage. Salary £4,232 p.a. (under review) plus USS superannuation. Apply quoting Ref. MN44A with curriculum vitae and the names of two referees to Assistant Bursar (Personnel), University of Reading, Whiteknights, Reading RG6 2AH. 2269(A)

### STUDENTSHIPS

## ROBERT GORDON'S INSTITUTE OF TECHNOLOGY, ABERDEEN

### SCHOOL OF PHARMACY

### RESEARCH STUDENTSHIP

Honours graduate, or student about to graduate, in Pharmacology, Pharmacy or related sciences for SRC (Case) Studentship in conjunction with Wellcome Research Laboratories to study intravascular platelet aggregation. CNA higher degree registration encouraged.

Applications or enquiries to:

Head of School of Pharmacy,  
Robert Gordon's Institute of Technology,  
Schoolhill,  
Aberdeen.  
AB9 1FR

2293(F)

## STUDENTSHIPS—continued

**THE UNIVERSITY OF SHEFFIELD  
DEPARTMENT OF METALLURGY  
SRC CASE STUDENTSHIPS**

Applications are invited from men and women for *ten* SRC/CASE studentships tenable from 1 October 1979 for research in the following fields:

- in co-operation with Delta Materials Research Limited, Ipswich. "Rheocasting of copper-based alloys"
- in co-operation with Firth Vickers Foundry Limited, Sheffield. "Processing and properties of centrispun castings"
- in co-operation with Wilkinson Match Limited, Slough. "Formation and properties of some high-strength glassy alloys"
- in co-operation with British Aluminium Company Limited, Gerrards Cross. "Stability of solid solutions extended by rapid freezing"
- in co-operation with Spring Research & Manufacturer's Association, Sheffield. "Effect of hot prestressing on reduction in spring relaxation"
- in co-operation with GKN Group Technological Centre, Wolverhampton. "The influence of texture on fatigue behaviour of steels"
- in co-operation with Johnson Matthey Research Centre, Reading. "High conductivity alloys produced by rapid solidification"
- in co-operation with CEGB Marchwood Laboratories, Southampton. "Creep damage at welds under complex stress"
- in co-operation with CEGB Scientific Services Division, Gravesend. "Void assessment in materials with changing structure"
- in co-operation with BSC Scunthorpe. "Mineralogy of sinters made from mixtures of home and imported ore"

Applicants with or expecting good Honours degrees in metallurgy, materials science or related fields should write to Professor G. J. Davies, Department of Metallurgy, University of Sheffield, Sheffield S1 3JD. Quote ref: R 321/G 2303(F)

**UNIVERSITY OF READING  
Department of Physiology & Biochemistry  
SRC STUDENTSHIP**

A three-year SRC Studentship is available from October 1979 to study the neurophysiology and neurochemistry of hypothalamic regulation of luteinising hormone release in the domestic fowl. A good honours degree will be required.

Applicants should send a curriculum vitae and names and addresses of two referees to Dr. R. T. Gladwell, from whom further details can be obtained (0734-85123, extension 7640).

**SRC-CASE Studentships in collaboration with the  
National Institute for Research in Dairying at Reading**

1. A three-year Studentship is available from October 1979 to study the hormonal control of lactation in ruminants. The project will include culture techniques to study biochemical changes during mammary differentiation using samples obtained from ruminants. A good Honours degree in biochemistry of related subjects will be required. The supervisors will be Professor R. Dils (University) and Dr. I. Forsyth (NIRD).

Applications, including curriculum vitae and names and addresses of two referees, should be sent as soon as possible to Professor Dils (0734-85123, extension 7600) or to Dr. Forsyth (0734-883103, extension 281) from whom further details can be obtained.

2. A three-year Studentship is available from October 1979 to study nutrient binding factors in mammary gland and milk. The project will involve the isolation, characterisation and biosynthesis of binding proteins for vitamin B<sub>12</sub>, folate and fatty acids. A good Honours degree in biochemistry or related subjects will be required. The supervisors will be Professor R. Dils (University) and Dr. M. Gurr (NIRD).

Applications, including curriculum vitae and names and addresses of two referees, should be sent as soon as possible to Professor Dils (0734-85123, extension 7600) or to Dr. Gurr (0734-85123, extension 260) from whom further details can be obtained. 2305(F)

**THE UNIVERSITY OF LEEDS  
DEPARTMENT OF PHYSICS**
**SRC STUDENTSHIP IN  
PHYSICS**

Applications are invited from persons holding, or expecting to obtain, a Class I or II (i) Honours degree in Physics for a SRC studentship leading to the degree of PhD in the above Department. The successful candidate will join an active group of polymer physicists working under the direction of Professor I. M. Ward.

Applications with curriculum vitae and the names and addresses of two referees should be sent to Professor G. J. Morgan, Department of Physics, University of Leeds, Leeds LS2 9JT, from whom further details may be obtained. 2302(F)

**THE UNIVERSITY OF  
LEEDS  
DEPARTMENT OF  
PLANT SCIENCES**

Applications are invited from persons who have, or expect to obtain, a first or upper second class honours degree (the course work for which has included the advanced study of plant pathology) for an SRC CASE studentship under the supervision of Dr Alec Carr of the Welsh Plant Breeding Institute and Mr Tom Preece of the University of Leeds. The project is concerned with resistance to *Xanthomonas graminis* infection in grasses.

Applications, with the names of two referees, should be sent to Mr T. F. Preece, Agriculture Building, The University of Leeds, Leeds LS2 9JT, by 1 August 1979. 2304(F)

**UNIVERSITY OF  
NOTTINGHAM MEDICAL  
SCHOOL  
RESEARCH STUDENTSHIP  
IN MICROBIOLOGY**

Applications are invited for a Research Studentship in Microbiology leading to the degree of PhD for work on the continuous monitoring of the action of antibiotics on dense bacterial cultures.

Applications with the names and addresses of two referees to Professor F. O'Grady, Department of Microbiology, University Hospital, Queen's Medical Centre, Nottingham NG7 2UH. 2291(F)

**UNIVERSITY OF  
ST ANDREWS**
**Department of Biochemistry  
and Microbiology  
MSc Studentship in  
Biochemistry**

Applications are invited for a MSc studentship financed by the Mental Health Foundation. The project will involve *in vivo* and *in vitro* studies on the mode of action of certain sulphur-containing compounds on pyridoxal phosphate-dependent enzymes of the GABA pathway in mammalian brain.

The one year studentship (with the possibility of a further two years' extension) will commence as soon as possible after 1st October, 1979. Candidates should hold, or expect to obtain in 1979, at least an upper second class honours degree in Biochemistry or a related subject.

Applications, together with the names and addresses of two referees, should be sent to—Dr R. Griffiths, Department of Biochemistry and Microbiology, University of St Andrews, Irvine Building, North Street, St Andrews, Fife. 2287(F)

**UNIVERSITY OF READING  
DEPARTMENT OF BOTANY  
SRC (CASE) STUDENTSHIP**

Applications are invited from good honours graduates in Plant Sciences for a studentship (in collaboration with the Glasshouse Crops Research Institute, Littlehampton) to investigate the properties of the plant photoreceptor, *phytochrome*, in green plant tissues.

Further information is obtainable from Dr. C. B. Johnson, Botany Department, Reading University, Whiteknights, Reading RG6 2AS, to whom applications should be sent before 13 July 1979. 2270(F)

**UNIVERSITY OF  
NEWCASTLE UPON TYNE  
DEPARTMENT OF NEUROLOGY**

A Postgraduate Research Studentship is available for three years from October 1st, 1979 at the Regional Neurological Centre. The work will be concerned with the enzymology of protein breakdown in muscle. There is a large unit studying various aspects of muscle diseases at the Regional Neurological Centre. Applicants should have or expect to obtain a good Honours degree with biochemistry or chemistry as a major subject, and the successful candidate will register for a PhD degree. Remuneration will be the same as that of postgraduate students supported by the Medical Research Council.

Applications, including the names and addresses of two referees should be sent, as soon as possible to Dr R. J. T. Pennington, Neurochemistry Department, Regional Neurological Centre, General Hospital, Newcastle upon Tyne NE4 6BE. (0632-738811.) 2343(F)

**SCOTT POLAR  
RESEARCH INSTITUTE  
(University of Cambridge)  
NERC Studentship**

Applicants with strong background in mathematics or physical sciences interested in interpretation of geophysical data are invited to study "Ice Sheet Dynamics derived from radio echo sounding and other data". The Institute's collection of radio echo sounding data from Antarctica built up over the past twelve years provides an outstanding opportunity for testing existing glaciological theory and developing new ideas to replace concepts shown to be inadequate.

Contact the Director, Telephone Cambridge 66499 ext 464 or write sending details. 2354(F)

**THE UNIVERSITY COLLEGE  
OF WALES  
ABERYSTWYTH  
DEPARTMENT OF ZOOLOGY  
SRC RESEARCH  
STUDENTSHIP**

Applications are invited from good Honours graduates in the biological sciences for a PhD research studentship funded by the Science Research Council. The applicant must have interests centred in research areas in cell and developmental biology, immunology, parasitology, animal physiology, or behaviour.

The studentship is tenable for three years starting October 1, 1979. Application, including a full curriculum vitae and the names of two referees, should be sent to Professor

B M Jones, Department of Zoology, University College of Wales, Aberystwyth, Dyfed, SW23 3DA. Requests for further information should be made to the Secretary at the same address. 2331(F)

## STUDENTSHIPS—continued

### THE UNIVERSITY COLLEGE OF WALES ABERYSTWYTH

**DEPARTMENT OF PHYSICS**  
Applications are invited for postgraduate work in the Department of Physics for Session 1979-80. The Science Research Council is prepared to offer to suitable candidates a limited number of Research Studentships for ionosphere and space research and studies of atomic collisions in plasmas, shock and detonation waves and the flow of non-Newtonian fluids.

Applications are also invited for one-year SRC Advanced Course Studentships for an MSc course on the Physics of the Upper Atmosphere.

Applications to Professor, Sir Granville Beynon, CBE, FRS, Department of Physics, University College of Wales, Aberystwyth, 2334(F)

### SCHOOL OF PHYSICS UNIVERSITY OF NEWCASTLE UPON TYNE RESEARCH STUDENTSHIPS IN GEOPHYSICS AND PLANETARY PHYSICS

PhD studentships are available for graduates with First or Upper Second Class Honours in either: Physics; Geophysics; Astronomy; Geology; Chemistry or Mathematics.

Current research interests are: Evolution of Planets; Fluid Dynamics; Geochronology; Geomagnetism; Geophysical Electronic Instrumentation; High Pressure Geophysics; History of Earth's Rotation; Palaeomagnetism; Plate Tectonics; Rift Systems; Rock and Mineral Magnetism; Study of Lunar Samples; Submarine Cable Currents.

For further particulars, please write as soon as possible, giving personal details, to Mr J. M. Walmsley, Administrative Assistant, School of Physics, The University, Newcastle upon Tyne NE1 7RU. 2351(F)

### Department of Paramedical Sciences

### Chemotherapy Research Unit

Applications are invited from graduates in biochemistry and/or microbiology for an SRC-CASE studentship to investigate electron transport mechanisms in the protozoan *Trichomonas vaginalis*, in conjunction with the Wellcome Laboratories of Tropical Medicine, Beckenham, Kent.

Candidates must possess a first or second class (upper) honours degree from a UK Institution and will be expected to register for a higher degree.

Applications, including a full curriculum vitae and the names of two referees should be sent as soon as possible to:

Head of Paramedical Sciences  
Department Ref. CF111  
North East London Polytechnic  
Romford Road  
London E15 4LZ  
Tel: 01-555 0811, Ext. 66,  
from whom further information may be obtained. 2332(F)

# NELP

### UNIVERSITY OF LIVERPOOL

#### DEPARTMENT OF DENTAL SURGERY

#### ELECTRON MICROSCOPE UNIT

A research studentship is available for candidates holding or expecting to gain a 1st or 2(1) honours degree in a biological discipline.

The research programme of the unit is concerned with investigating the utilization of calcium and other elements in hard tissue forming cells by using electron microscopical analytical techniques.

Applications together with the names of two referees should be received as soon as possible by The Registrar, The University, PO Box 147, Liverpool L69 3BX. Quote Ref: RV/668. 2307(F)

### THE UNIVERSITY COLLEGE OF WALES ABERYSTWYTH DEPARTMENT OF AGRICULTURAL BOTANY MAFF STUDENTSHIP SYMBIOTIC NITROGEN FIXATION

Applications are invited from suitably qualified candidates to work on the ecology of *Rhizobium trifolii* with particular reference to the use of genetic markers and the ELISA technique.

The successful candidate must have at least an Upper Second Honours degree in Agricultural Botany or Microbiology and will be expected to study for a higher degree under the supervision of Dr D Gareth Jones. The ability to drive a car is desirable.

Application forms are available from the Registrar, University College of Wales, Aberystwyth, Dyfed. Closing date for applications 14 July 1979. 2330Z(F)

### THAMES POLYTECHNIC SCHOOL OF BIOLOGICAL SCIENCES

#### SRC CASE STUDENTSHIP

Applications are invited for a CASE studentship to undertake an investigation into the role of *Pseudomonas morsprunorum* lipopolysaccharide in cancer disease of cherries. The award is tenable from 1 October 1979.

Applicants should hold (or expect to obtain) a good honours degree in biochemistry or chemistry, and if in the latter, should preferably have some experience in microbiology. The investigation will be carried out in collaboration with East Malling Research Station, Maidstone, Kent, and will be jointly supervised by Dr A. R. W. Smith (Thames Polytechnic) and Dr R. C. Hignett (East Malling Research Station).

Further particulars and form of application may be obtained from the Staffing Officer, Thames Polytechnic, Wellington Street, London, SE18 6PF, to whom completed applications should be returned by 10 July 1979. 2285(F)

### SRC STUDENTSHIPS IONOSPHERIC RADIO WAVE PROPAGATION

Applications are invited for SRC CASE studentships leading to the PhD degree. The research programme involves basic studies in ionospheric physics, together with investigations of how this medium affects the performance of radio navigation and communications systems. The more applied topics involve considerable use of microprocessors.

Further details of these projects can be obtained from Dr. T. B. Jones, Physics Department, The University, Leicester LE1 7RH. 2317(F)

### PORTSMOUTH POLYTECHNIC

#### DEPARTMENT OF BIOLOGICAL SCIENCES

#### SRC CASE Studentships

Post 1 Title: The use of tree-rings as a means of dating timbers from historical sites.

Supervisor: Dr F. A. Hibbert.

Co-operating body: Department of Environment, Ancient Monuments Laboratory, London.

Post 2 Title: Microbial infestation of Diesel fuel storage delivery systems. Supervisors: Drs R. A. Eaton and E. B. G. Jones.

Co-operating body: Ministry of Defence, Central Dockyard Laboratory, Portsmouth.

#### SRC Studentship

Post 3 Title: Oogenesis in developmentally retarded tadpoles of *Xenopus laevis*.

Supervisor: Dr S. C. Turner.

Title: Some aspects of the biology of the larval stage of digeneans.

Supervisor: Dr T. A. J. Reader.

#### SRC Advanced Course

#### Studentships MSc COURSE IN THE BIODETERIORATION OF MATERIALS

A number of SRC awards are available for suitably qualified students who wish to apply for the above course.

Starting date for all posts October 1, 1979.

Applications to include a curriculum vitae and the names of two referees should be sent to the Administrative Assistant, Department of Biological Sciences, Portsmouth Polytechnic, King Henry I Street, Portsmouth PO1 2DY from whom further information can be obtained if desired.

Closing date for all applications: June 28, 1979. 2338(F)

### SCHOOL OF CHEMISTRY CASE AWARDS

Applications are invited for SRC/CASE studentships in the following areas of research:

a) "The Chemistry of Dihalogenocarbene Adducts of Terpenes" under the supervision of Dr. M. S. Baird and in collaboration with Bush Boake Allen Ltd.  
b) "Testing of pH glass electrodes at high temperatures" (Dr. A. K. Covington in association with Dr. P. O. Kane, ICI Mond Division, Runcorn, Cheshire). The project will be concerned with the extension of a new method of testing glass electrodes to higher temperatures (up to 130°C). It will provide a good training in industrial use and appraisal of electrochemical sensing devices.

c) "Spectroscopic Studies of Ammonium Urates". The project, to be carried out with UKAEA as the co-operating body, is designed to develop an understanding of the chemistry of the ammonium urate system. This understanding will assist the use of ammonium urate for the removal of uranium from solution. The study will involve spectroscopic studies of ammonium urates in particular using X-ray photoelectron spectroscopy. Under the supervision of Dr. P. M. A. Sherwood.  
d) "The Electrode Kinetics of Electron Exchange Reactions". The project is to study, under the supervision of Dr. J. A. Harrison, the reactions with metal ion complexes, metal deposition and electrocrystallisation phenomena at interfaces using the latest automated equipment. The kinetic information will be used to make correlations and predictions in allied technological fields (in conjunction with the International Nickel Co.).

All successful applicants will be registered for a PhD degree and grants will be in accordance with the current SRC Regulations.

Letters of application including the names of two referees should be sent to Mr. S. J. Hall, School of Chemistry, The University of Newcastle upon Tyne, NE1 7RU. 2277(F)

# university college of swansea

#### CASE Research Studentships

Applications are invited from candidates with upper second class honours degrees or equivalent, or from those expecting to obtain such a degree this Summer, for the following SRC (CASE Research Studentships) in the Department of Chemistry: (a) the use of borane-amine complexes in organic synthesis (Professor A. Pelter, in collaboration with ICI Pharmaceuticals Ltd.); (b) the synthesis of dihydroisocoumarins related to berberine (Dr. R. S. Ward, in collaboration with May and Baker Ltd.); (c) the synthesis of novel macrocyclic lactones with potential nervous system activity (Dr K. Smith, in collaboration with Pfizer Ltd.). Industrial grants are also available for work on gas chromatography and analytical chemistry. The value of the studentships will be in line with SRC awards. Further particulars may be obtained from Professor A. Pelter, Department of Chemistry, University College of Swansea, Singleton Park, Swansea, SA2 8PP. 2314(F)

### AGRICULTURAL RESEARCH COUNCIL STUDENTSHIP

Applications are invited for a studentship to work in the Protein Section of the Meat Research Institute.

The project is to investigate the turnover and remodelling of the connective tissues in relation to growth and development.

This will consist of radioactive incorporation studies together with autoradiography, the chemical analysis of connective tissue components and an examination of the cellular mechanisms for resorption of tissue.

The project is expected to form the basis for a PhD thesis in the University of Bristol and the appointment will commence in October, 1979.

Candidates should possess a First or Upper Second Class degree in Biochemistry.

Further particulars of the award and allowances together with Application Forms may be obtained from the Personnel Officer, Agricultural Research Council, Meat Research Institute, Langford, Bristol, BS18 7DY. 2347(F)

### SRC STUDENTSHIP DEPARTMENT OF PHARMACEUTICS

Applications are invited from graduates with first class or upper second class honours degrees in pharmacy or related disciplines for an SRC studentship for research in one of the following areas.

Solution properties of surfactants.

Activity of liposomes containing antibiotics.

Cell and tissue culture.

Please write stating interests to Professor A. T. Florence, Department of Pharmaceutics, University of Strathclyde, Glasgow G1 1XW.

2301(F)



## STUDENTSHIPS—continued

QUEEN MARY COLLEGE  
(University of London)SRC CASE  
RESEARCH STUDENTSHIP

Applications are invited for a research studentship, tenable for three years from October 1979, leading to the degree of MPhil/PhD of the University of London. The project is on the control of amylase production and secretion in yeast and will be jointly supervised by Professor E. A. Bevan and Dr Ivor Evans. It will involve physiological investigations as well as the application of advanced techniques of molecular genetics.

Candidates with, or expecting to gain, a first or upper second class honours degree in an appropriate subject should apply at once to Professor E. A. Bevan, Department of Plant Biology & Microbiology, Queen Mary College, Mile End Road, London E1 4NS, from whom further particulars can be obtained.

2345(F)

UNIVERSITY COLLEGE  
LONDON  
DEPARTMENT OF PHYSICS  
AND ASTRONOMY  
MULLARD SPACE SCIENCE  
LABORATORY  
Holmbury St. Mary  
Dorking Surrey

Applications are invited for a post-doctoral Research Assistantship financed by the Science Research Council for work on instrument development and on the analysis and interpretation of data in either of the Laboratory's two areas of research — X-ray astronomy and magnetospheric physics. The appointment will be for three years, commencing as soon as possible. Salary within the range of £4232 to £6627 plus USS membership.

Applications, with curriculum vitae and the names of two referees, should be sent to Professor R.L.F. Boyd, C.B.E., F.R.S., at the above address. 2366(P)

SUNDERLAND  
POLYTECHNIC  
FACULTY OF SCIENCE  
SCHOOL OF PHARMACY  
RESEARCH ASSISTANTS

Applications are invited from graduates possessing good honours degrees in pharmacy or other appropriate disciplines, or from undergraduates expecting to obtain such degrees this year. The successful applicants will be expected to work for a higher degree (MPhil/PhD) in one of the following fields:

- P1 — The effect of surfactant adsorption on drug release.
- P2 — The synthesis of potential drug molecules based on phloroglucinol.
- P3 — An investigation of the preparation and chemical, physical, and biological properties of phrazolopyridines.
- P4 — Chemotaxonomic and phytochemical investigations in the sub-family Aurantioideae (Rutaceae).
- P5 — A study of the migration of particles and droplets in a shear field.

The salary for an assistantship, which is normally tenable for up to three years, is £2,778 to £3,054 per annum (under review).

Applicants are requested to state the post(s) in which they are interested.

An application form and further details may be obtained from the Personnel Officer, Sunderland Polytechnic, Chester Road, Sunderland, SR1 3SD, and should be returned as soon as possible.

2267(P)

THE UNIVERSITY  
OF SHEFFIELDRESEARCH ASSISTANTSHIP  
IN  
THEORETICAL CHEMISTRY

The University expects shortly to be in a position to appoint a Postdoctoral Research Assistant, with support from the Science Research Council, for work in the general area of quantum chemistry with emphasis on Green's function and density functional methods. Appointment will be on Range 1A at up to £4910 p.a. Tenable three years subject to annual renewal, from 1 October 1979. Further information from Professor R. McWeeny, Department of Chemistry, the University, Sheffield S3 7HF. Quote ref: R327/G. 2358(P)

Applications are invited from suitably qualified science or dental graduates to work for a higher degree in one of the following fields of study currently in progress in the Department of Oral Biology, University of Alberta, Edmonton, Alberta, Canada.

Developmental Biology

Neurophysiology

Biochemistry of Connective Tissues

Calcium regulating hormones

Tumor Development

Graduate assistantships can be awarded to successful applicants.

Applicants can be directed to Dr. J. W. Osborn, Chairman, Department of Oral Biology, University of Alberta, Edmonton, Alberta, T6G 2N8, Canada. W190(P)

BIOCENTER  
(University of Basel)  
POSTDOCTORAL  
RESEARCH  
ASSISTANTSHIPS

One position is available immediately and one in mid-1980 to investigate mechanisms of *in ovo* oncogenesis induced by avian tumour viruses using various approaches to identify the *in vivo* target cells and the biochemical mechanisms of tumor induction. We are particularly interested in the infection of the chick embryo by tumor viruses in the early stages of embryonic development. Persons having experience in the area of modern embryology of the chick or mouse, using immunological, biochemical, histochemical or transplant technology will be preferred. Interested candidates please send curriculum vitae, bibliography, and the names of two referees to Prof. R. M. Franklin, Biocenter, University of Basel, Klingelbergstrasse 70, CH-4056 Basel/Switzerland. W194(P)

UNIVERSITY OF LONDON  
KING'S COLLEGE  
DEPARTMENT OF BIOPHYSICS  
RESEARCH  
ASSISTANTSHIP

Applications are invited for the post of research assistant to work on a Cancer Research Campaign — supported project concerned with the interactions of carcinogens and anti-cancer agents with nucleic acids. A degree in biochemistry, chemistry or related fields is required; experience in separation methods and/or handling of nucleic acids would be useful. Starting salary in the range of £3,775 to £4,333 + £502 London allowance. Appointment is for one year in the first instance, starting from October 1979, with the possibility of renewal for a further two years. Applications, with a curriculum vitae and names of two referees, to Dr S. Neidle, Department of Biophysics, King's College, 26-29 Drury Lane, London WC2B 5RL, from whom further particulars may be obtained. 2337(P)

## APPOINTMENTS WANTED

BIOLOGIST/MOLECULAR GENETICIST 26, Cambridge First, PhD Edinburgh in molecular genetics. Experienced in P3 recombinant DNA work, marine and terrestrial ecology (publications), humanistic psychology, amateur astronomy. Well travelled, seeks imaginative employment anywhere. Nick Hanscomb, Lamancha House, Lamancha, Peeblesshire. 2294(B)

## FELLOWSHIPS

UNIVERSITY OF  
NOTTINGHAM  
SCHOOL OF AGRICULTURE  
Sutton Bonington,  
Nr. LoughboroughDEPARTMENT OF PHYSIOLOGY &  
ENVIRONMENTAL STUDIES

Research opportunities in  
Environmental Science

1. A fellowship is available for a project supported by the ARC. This is concerned with the physics and economics of very low cost solar energy collectors for use in crop drying and the successful candidate will work with Dr. J. A. Clark and Mr. B. Wilton.

The appointment is for 3 years. Candidates should preferably have a PhD or equivalent experience in an appropriate branch of physics, engineering, or environmental science (salary scale commencing £4,776 p.a. at age 26). Applications will also be considered from newly qualified graduates with a good honours degree (salary scale commencing at £3,689 p.a.).

2. The Heat Balance of Wet Vegetation.

The duration of leaf wetness is an important factor influencing the development of plant diseases and the uptake of soluble pollutant gases. A PhD studentship is available for a study of micrometeorological processes which determine leaf wetness and to develop computer simulation models. A good honours degree in physics or environmental science is desirable. Candidates will work with Dr. M. H. Unsworth.

Further information may be obtained from the member of staff referred to and applications including a full curriculum vitae with the names and addresses of two referees should be submitted to them at the School as soon as possible. 2274(E)

POSTDOCTORAL  
FELLOWSHIPSTHE ROCHE INSTITUTE  
OF MOLECULAR BIOLOGY

Unusual opportunities are offered by the Roche Institute of Molecular Biology for postgraduate training and education. These postdoctoral fellowships are in the field of biochemistry, genetics, virology, neurobiology, pharmacology, and other areas of molecular biology. The Roche Institute, under the direction of Dr Sidney Udenfriend, is dedicated to basic research, and approximately 30 staff members enjoy independence in their choice and pursuit of research problems. Postdoctoral positions are now available in the departments of Physiological Chemistry & Pharmacology (Dr Sydney Spector, Chairman), Biochemistry (Dr Herbert Weissbach, Chairman), and Cell Biology (Dr Arthur Weissbach, Chairman). Fellowships at the Institute, which is located in Nutley, New Jersey, about 10 miles west of New York City, are awarded on an equal opportunity basis to recent recipients of an M.D., a Ph.D. or an equivalent degree in the biological or biochemical sciences. Stipends start at \$14,500 plus benefits. For further information, contact Dr George J. Cardinale, Roche Institute of Molecular Biology, Nutley, New Jersey 07110.

W195(E)

UNIVERSITY OF  
ST. ANDREWS  
RESEARCH FELLOWSHIP  
IN LASER PHYSICS

There is a vacancy for a Postdoctoral Research Fellow to work on a CO<sub>2</sub> TEA laser contract. Previous experience in laser physics and high vacuum technology would be an advantage.

Starting salary within the range £4,333 to £4,910 per annum (under review) plus USS; the appointment is tenable for two years in the first instance from October 1979.

Applications including a curriculum vitae and the names of two referees should be sent as soon as possible to Dr. A. L. S. Smith, Physics Department, University of St. Andrews, St. Andrews, Fife KY16 9SS, from whom further details can be obtained. 2295(E)

## EMBO

## European Molecular Biology Organization

LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY  
AUTUMN 1979 AWARDS

Next deadline: August 31, 1979

EMBO long term fellowships are initially awarded for one year. Applications for a renewal for a second year and subsequently in cases of exceptional scientific merit for a third year are considered.

To be eligible a candidate must hold a doctors degree. Preference will be given to European and Israeli applicants wishing to work within Europe or Israel. EMBO long term fellowships are not, however, awarded for exchanges between laboratories within any one country. Applications for fellowships to be held outside Europe and Israel are considered but they have a lower priority, as do applications from non-European scientists wishing to work in Europe or Israel.

Successful applicants will be notified of their awards on October 29, 1979.

Further details and application forms may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, 69 Heidelberg 1, Postfach 1022.40, F. R. Germany.

W188(E)

## FELLOWSHIPS continued

LIVERPOOL  
SCHOOL OF  
TROPICAL MEDICINE

Department of Parasitology

Applications are invited for a POSTDOCTORAL FELLOWSHIP to work on cultivation *in vitro* and moulting processes of filarial worms. A biochemical background with some experience of tissue culture techniques is desirable for this post. Appointment for one year to commence immediately. Starting salary £4,232 per annum, USS benefit.

Applications in the form of curriculum vitae and the names of two referees should be sent as soon as possible to the Head of Department of Parasitology, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA.

2363(E)

UNIVERSITY OF EXETER  
CHEMISTRY DEPARTMENT  
Structural Studies  
of Liquid Crystals

Applications are invited for a Postdoctoral Research Fellowship to work with Professor A J Leadbetter on X-ray diffraction studies of smectic liquid crystals. Crystal structure determinations of the crystalline precursors and some neutron scattering work might also be involved depending on the interests and experience of the person appointed. Applicants may be chemists or physicists and some experience of X-ray diffraction and computing would be an advantage, though not essential.

The post is tenable for one year in the first instance with the possibility of renewal for a further year. Starting date by arrangement on or after 1 September 1979 at a point on the salary scale commencing at £4,333 pa, depending on age and experience.

Applications should be sent as soon as possible to Professor A J Leadbetter, Department of Chemistry, Stocker Road, Exeter.

2311(E)

## UNIVERSITY OF BRISTOL

VETERINARY  
RESEARCH FELLOWSHIP

Applications are invited from veterinary graduates for a Research Fellowship to investigate the physiology of cervical dilatation in the ewe. The successful applicant will join a research group in the Department of Anatomy which is currently working on problems of the myometrium and the physiology of relaxin. The group has accommodation for sheep at Bristol and at the School of Veterinary Science, Langford. It is intended that the applicant will employ a variety of research techniques in the elucidation of this problem, including chronic recording of cervical softening *in vivo*. The opportunity exists to investigate the histochemistry of the glycosaminoglycans of the cervix. In addition, the Department offers a wide spectrum of facilities and expertise in electron-microscopy, electro-physiology, polypeptide biochemistry, neurophysiology, hard tissue studies, and experimental surgery.

The Fellowship is provided by the Agricultural Research Council and awards a salary of £4,232 to £8,452 depending upon age and experience. The appointee will be expected to register for a higher degree.

Applications in duplicate which should include a full curriculum vitae together with the names and addresses of two referees should be sent to Professor D. G. Porter, Pre-Clinical Veterinary Studies, Department of Anatomy, The Medical School, University of Bristol, Bristol BS8 1TD.

2248(E)

ALL SOULS COLLEGE  
RESEARCH FELLOWSHIP

The College intends to make an election to a Research Fellowship in the course of the academic year 1979-80. It is anticipated that the election will be made in or about February 1980.

The Research Fellowship will be tenable for a period of seven years. The College would normally renew the Fellowship if there is evidence of satisfactory achievement. The retiring age is 67. The stipend payable is related to the age of the Fellow upon appointment.

The Fellowship will be open to those working in the following fields of study: Law, History, Philosophy, Politics, Economics, English, Classics and Mathematical Sciences.

The Fellowship is open to men and women. (It is assumed that Privy Council approval will shortly be forthcoming for an amendment to the Statutes opening membership of the College to women.)

Further particulars, including salary and terms of appointment, may be obtained from The Warden's Secretary, All Souls College, Oxford. Applications, which should contain the names of not more than three referees, must reach the Warden not later than 1 October 1979, and should be marked "Research Fellowship".

2282(E)

THE UNIVERSITY OF HULL  
DEPARTMENT  
OF APPLIED PHYSICS

Applications are invited for two research fellowships, which are immediately available, for a period of up to three years.

Department of Applied Physics 1. A research fellow is required to measure the frequency characteristics of the current generation of pulsed CO<sub>2</sub> lasers and to devise ways of improving their frequency stability using techniques such as hybridisation and injection locking.

2. A research fellow is required to work on the use of tunable multi-atmosphere, molecular gas lasers for the remote sensing of trace gases in the atmosphere.

These fellowships are suitable for graduates in Physics, Applied Physics or Electronic Engineering with at least three years research experience in relevant fields.

Salary: At an appropriate point on the scale £4,333 to £7,521 per annum. (From 1st October)

Applications should give full details of education, qualifications and research experience, together with the names of two referees, and should be sent by 27th July 1979 to Dr. E. L. Thomas, Department of Applied Physics, the University of Hull, Hull, HU6 7RX from whom further details may be obtained.

2350(E)

RESEARCH FELLOWSHIP  
IMPERIAL CANCER  
RESEARCH FUND

We have a vacancy for a post-doctoral Research Fellow to join a group working on ageing and neoplastic transformation in epithelial cell systems *in vitro*. Special experience in cell culture techniques, cell hybridisation, etc., an advantage.

Appointment will be for three years. Salary according to qualifications and experience.

Further information from Dr L. M. Franks (01-242 0200, ext. 211). Applications with curriculum vitae and names of two referees should be sent to The Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX.

2250(E)

LA TROBE UNIVERSITY  
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LA TROBE

## RESEARCH FELLOWSHIP

Applications are invited for a La Trobe Research Fellowship. Applicants should be at the recent (within the last five years) postdoctoral level and the Fellowship will normally be awarded to an applicant with research experience away from La Trobe University. The successful applicant must hold a doctoral degree or have equivalent qualifications at the time of commencement of the Fellowship.

The Fellowship will be awarded initially for a period of 12 months and may be renewed for a further maximum period of 12 months. Applicants must be able to take up the Fellowship no later than 30 June 1980.

Salary is \$A15,786 per annum.

Further information and application forms are available from the Graduate Studies Officer. Enquirers should indicate the research discipline of interest to them. The closing date for applications is 31 August 1979. D D Neilson, Registrar, Bundoora, Victoria 3083, Australia.

2321(E)

## CONFERENCES

EUROPEAN SOCIETY FOR  
COMPARATIVE SKIN  
BIOLOGY

Symposium on Surface Eco-systems, Ayr, Scotland, September 13, 14, 1979. International Conference, including symposium on Insect Cuticle, Copenhagen, August 25-28, 1980. Details from Dr P. E. Budtz, August Krogh Institute, 13 Universitetsparken, Copenhagen, Denmark.

2281(C)

## ASSOCIATESHIPS

UNIVERSITY OF  
CAMBRIDGEDEPARTMENT OF THEORETICAL  
CHEMISTRY

A post-doctoral research associateship is available for two years to develop a new method of computing correlated relativistic atomic wavefunctions with the aim of investigating the electronic structure of actinide and superheavy elements. Applications including a curriculum vitae and the names and addresses of two referees should be sent as soon as possible to Dr N. C. Pyper, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW.

2336(O)

## COURSES

UNIVERSITY OF LEICESTER  
MSc in Experimental  
Space Physics

Applications are now being considered for the Space Physics course for entry in October. Candidates should have a degree with 1st or 2nd class honours in Physics, Mathematics or Engineering. The course runs for 12 months and includes lectures, dissertation and project work covering both scientific (astronomy, earth sciences applications) and technical (propulsion orbits, communications, etc) aspects of present-day space research. A number of SRC studentships are available to successful applicants.

Write for further details, including the names and addresses of two academic referees, to Professor K A Pounds, Department of Physics, University of Leicester, Leicester LE1 7RH.

2283(D)

## AWARDS

IMPERIAL  
COLLEGE

DEPARTMENT OF CHEMISTRY SRC/CASE awards from October 1st 1979, are available for suitably qualified graduates in the following areas, with the supervisors and industrial sponsors indicated:

1. Development of modified electrodes for electrocatalysis (Professor W. J. Albery, I.C.I., Mond Division).
2. Development of electrochemical sensors for power station waters (Professor W. J. Albery: Central Electricity Research Laboratory).
3. Application of microprocessors to electroanalytical techniques (Professor W. J. Albery: Laboratory of the Government Chemist).
4. Alkali-metal peroxy-salts (Drs. W. P. Griffith and A. C. Skapski: Interlox Chemicals Ltd.).
5. Absorption of organic molecules in zeolites from liquid-phase solutions (Dr. L. V. C. Rees: Laporte Industries Ltd.).
6. Binary and ternary ion-exchange equilibria and kinetics in zeolites (Dr. L. V. C. Rees: Procter and Gamble Ltd.).

Applications should be addressed to the supervisors named, at Chemistry Department, Imperial College, London SW7 2AY, as soon as possible: telephone enquiries to 01-589 5111 are welcome.

2322(N)

## LECTURES

THE  
EXPERIMENTAL PSYCHOLOGY SOCIETY

The Seventh Sir Frederick Bartlett Lecture will be delivered by Professor M. I. Posner, of the Department of Psychology, University of Oregon.

## ORIENTING OF ATTENTION

5.30 pm, Thursday July 5th, 1979.

Main Lecture Theatre,  
Department of Experimental Psychology,  
University of Oxford.

The lecture will be open to the public.

2360(K)

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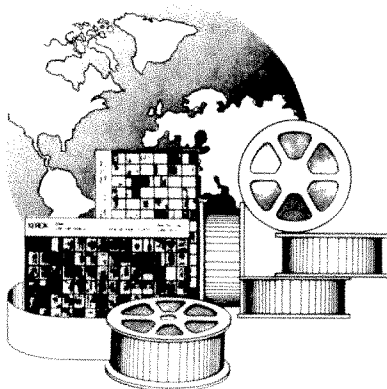
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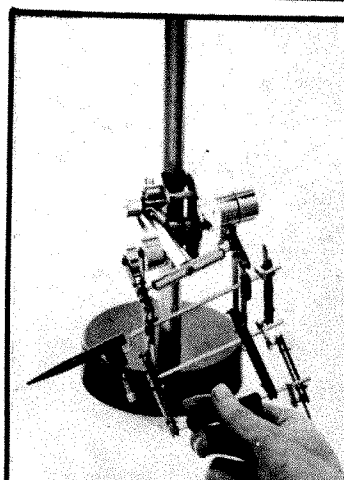
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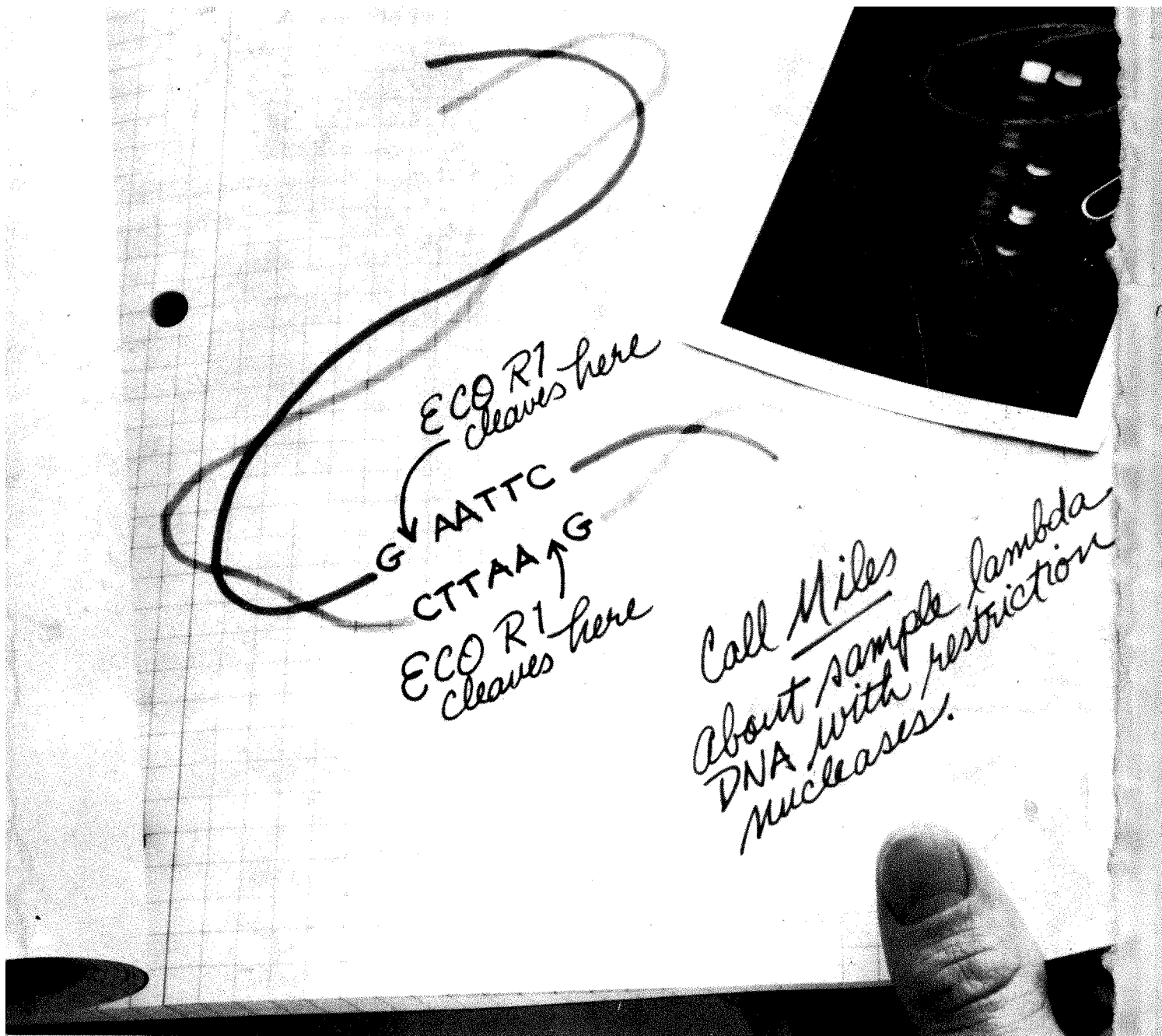
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